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(71) Applicant: HESKA CORPORATION [US/US]; 1825 Sharp Point Drive, Fort Collins, CO 80525 (US).

(72) Inventors: FRANK, Glenn, Robert; 10317 North County Road 13, Wellington, CO 80549 (US). RUSHLOW, Keith, E.; 1600 Dogwood Court, Fort Collins, CO 80525 (US).

(74) Agents: HANLEY, Elizabeth, A. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).

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(54) Title: METHOD TO DETECT CANINE IgE

(57) Abstract

The present invention includes a method to detect canine IgE using a canine Fc epsilon receptor (FcεR) to detect canine IgE antibodies in a biological sample from a canid. The present invention also relates to kits to perform such methods.

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METHOD TO DETECT CANINE IgE

Field of the Invention

The present invention relates to a novel method to detect canine epsilon immunoglobulin (IgE). The present invention also includes novel kits to detect canine IgE as well as methods to produce the detection reagent.

Background of the Invention

Diagnosis of disease and determination of treatment efficacy are important tools in medicine. In particular, detection of IgE production in an animal can be indicative of disease. Such diseases include, for example, allergy, atopic disease, hyper IgE syndrome, internal parasite infections and B cell neoplasia. In addition, detection of IgE production in an animal following a treatment is indicative of the efficacy of the treatment, such as when using treatments intended to disrupt IgE production.

Until the discovery of the present invention, detection of IgE in samples obtained from non-human animals has been hindered by the absence of suitable reagents for detection of IgE. Various compounds have been used to detect IgE in IgE-containing compositions. In particular, antibodies that bind selectively to epsilon idiotype antibodies (i.e., anti-IgE antibodies) have been used to detect IgE. These anti-IgE antibodies, however, can cross-react with other antibody idiotypes, such as gamma isotype antibodies. The discovery of the present invention includes the use of a canine Fc epsilon receptor ($Fc_\epsilon R$) molecule to detect the presence of IgE in a putative IgE-containing composition. Canine high affinity $Fc_\epsilon R$ consists of three protein chains, alpha, beta and gamma. Hayashi et al. have disclosed the nucleic acid sequence for the alpha chain (GenBank Accession No. D16413, submitted June 8, 1993). A canine $Fc_\epsilon R$ molecule provides an advantage over, for example anti-IgE antibodies, to detect IgE because a canine $Fc_\epsilon R$ molecule can bind to a canine IgE with more specificity (i.e., less idiotype cross-reactivity) and more sensitivity (i.e., affinity) than anti-IgE binding antibodies.

Thus, methods and kits are needed in the art that will provide specific detection of canine IgE using canine $Fc_\epsilon R$.

Summary of the Invention

The present invention includes detection methods and kits that detect canine IgE.

One embodiment of the present invention is a method to detect canine IgE comprising: (a) contacting an isolated canine Fc_ϵ receptor ($Fc_\epsilon R$) molecule with a putative canine IgE-containing composition under conditions suitable for formation of a $Fc_\epsilon R$ molecule:IgE complex; and (b) determining the presence of IgE by detecting the $Fc_\epsilon R$ molecule:IgE complex, the presence of the $Fc_\epsilon R$ molecule:IgE complex indicating the presence of IgE. In particular, the canine $Fc_\epsilon R$ molecule comprises at least a portion of a $Fc_\epsilon R$ alpha chain that binds to canine IgE.

Another embodiment of the present invention is a method to detect canine flea allergy dermatitis comprising: (a) immobilizing a flea allergen on a substrate; (b) contacting the flea allergen with a putative canine IgE-containing composition under conditions suitable for formation of an antigen:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain antigen:IgE complex binding to the substrate; and (d) detecting the presence of the antigen:IgE complex by contacting antigen:IgE complex with a canine $Fc_\epsilon R$ molecule. In particular, the flea allergen is a flea saliva antigen.

The present invention also includes a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a canine Fc_ϵ receptor ($Fc_\epsilon R$) molecule and a means for detecting canine IgE. Another embodiment is a kit for detecting flea allergy dermatitis comprising a canine Fc_ϵ receptor ($Fc_\epsilon R$) molecule and a flea allergen.

Detailed Description of the Invention

The present invention relates to the discovery that purified high affinity canine Fc epsilon receptor (i.e., $Fc_\epsilon RI$; referred to herein as $Fc_\epsilon R$) can be used in canine epsilon immunoglobulin (referred to herein as IgE or IgE antibody)-based detection (e.g., diagnostic, screening) methods and kits. The use of canine $Fc_\epsilon R$ in diagnostic methods and kits is unexpected because the use of canine $Fc_\epsilon R$ avoids complications presented by use of antibodies that bind to IgE (i.e., anti-IgE antibodies). Such complications include, for example, non-specific binding of anti-IgE antibodies to other classes of immunoglobulin such as gamma immunoglobulin (i.e., IgG).

One embodiment of the present invention is a method to detect a canine IgE using an isolated canine $Fc_\epsilon R$ molecule. It is to be noted that the term "a" entity or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. Furthermore, a compound "selected from the group consisting of" refers to one or more of the compounds in the list that follows, including mixtures (i.e., combinations) of two or more of the compounds.

According to the present invention, an isolated, or biologically pure, $Fc_\epsilon R$ molecule, is a molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated canine $Fc_\epsilon R$ molecule of the present invention can be obtained from its natural source (e.g., from a canine mast cell), can be produced using recombinant DNA technology or can be produced by chemical synthesis.

A $Fc_\epsilon R$ molecule (also referred to herein as $Fc_\epsilon R$ or $Fc_\epsilon R$ protein) of the present invention can be a full-length protein, a portion of a full-length protein or any homolog of such a protein, wherein the $Fc_\epsilon R$ molecule is capable of binding specifically to IgE. As used herein, a protein can be a polypeptide or a peptide. A $Fc_\epsilon R$ molecule of the present invention can comprise a complete $Fc_\epsilon R$ (i.e., alpha, beta and gamma $Fc_\epsilon R$ chains), an alpha $Fc_\epsilon R$ chain (also referred to herein as $Fc_\epsilon R$ α chain) or portions thereof. Preferably, a $Fc_\epsilon R$ molecule comprises at least a portion of a $Fc_\epsilon R$ α chain that binds to IgE, i.e., that is capable of forming an immunocomplex with an IgE constant region.

An isolated canine $Fc_\epsilon R$ molecule of the present invention, including a homolog, can be identified in a straight-forward manner by the $Fc_\epsilon R$ molecule's ability to form an immunocomplex with a canine IgE. Examples of $Fc_\epsilon R$ homologs include $Fc_\epsilon R$ proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog includes at least one epitope capable of forming an immunocomplex with an IgE.

$Fc_\epsilon R$ homologs can be the result of natural allelic variation or natural mutation. $Fc_\epsilon R$ homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

According to the present invention, a preferred canine $Fc_\epsilon R$ α chain of the present invention is encoded by at least a portion of the nucleic acid sequence of the coding strand of a cDNA encoding a full-length $Fc_\epsilon R$ α chain protein represented herein as SEQ ID NO:19, the portion at least encoding the IgE binding site of the $Fc_\epsilon R$ α chain protein. Other suitable canine $Fc_\epsilon R$ α chains useful in the present invention include those described herein in the Examples section. The double-stranded nucleic acid molecule including both the coding strand having SEQ ID NO:19 and the complementary non-coding strand (the nucleic acid sequence of which can be readily determined by one skilled in the art and is shown herein as SEQ ID NO:21) is referred to herein as $Fc_\epsilon R$ nucleic acid molecule $ncFc_\epsilon R\alpha 4_{991}$. Translation of SEQ ID NO:19 suggests that nucleic acid molecule $ncFc_\epsilon R\alpha 4_{991}$ encodes a full-length $Fc_\epsilon R$ α chain protein of about 253 amino acids, referred to herein as $PcFc_\epsilon R\alpha 4_{253}$, represented by SEQ ID NO:20, assuming an open reading frame having an initiation (start) codon spanning from about nucleotide 35 through about nucleotide 37 of SEQ ID NO:19 and the termination codon spans from about nucleotide 793 through about nucleotide 795 of SEQ ID NO:19. The coding region encoding $PcFc_\epsilon R\alpha 4_{253}$, excluding the stop codon, is represented by nucleic acid molecule $ncFc_\epsilon R\alpha 4_{759}$, having a coding strand with the nucleic acid sequence represented by SEQ ID NO:22 and a complementary strand with nucleic acid sequence SEQ ID NO:23. SEQ ID NO:19 encodes a signal peptide spanning from about amino acid 1 through about amino acid 24, as well as a mature protein of about 229 amino acids, denoted herein as $PcFc_\epsilon R\alpha 4_{229}$, the amino acid sequence of which is represented herein as SEQ ID NO:24. The nucleic acid molecule encoding the apparent mature protein is referred to as $ncFc_\epsilon R\alpha 4_{687}$, the nucleic acid sequence of the coding strand of which is denoted herein as SEQ ID NO:30. SEQ ID NO:19 also encodes a hydrophobic transmembrane domain which extends from about amino acid 172 to about amino acid 228 of SEQ ID NO:24. Knowledge of these nucleic

acid and amino acid sequences allows one skilled in the art to make modifications to the respective nucleic acid molecules and proteins to, for example, develop a canine $Fc_\epsilon R$ α chain protein with increased solubility and/or a truncated protein capable of detecting canine IgE, e.g., $PcFc_\epsilon R\alpha 4_{197}$, spanning from about amino acid 1 to about amino acid 197 of SEQ ID NO:20, and having SEQ ID NO:28; or $PcFc_\epsilon R\alpha 4_{173}$, spanning from about amino acid 25 to about amino acid 197 of SEQ ID NO:20, and having SEQ ID NO:31.

Preferred $Fc_\epsilon R$ molecules include $PcFc_\epsilon R\alpha 4_{253}$, $PcFc_\epsilon R\alpha 4_{229}$, $PcFc_\epsilon R\alpha 4_{197}$, $PcFc_\epsilon R\alpha 4_{173}$ and allelic variants thereof, as well as $PcFc_\epsilon R\alpha 1_{197}$, $PcFc_\epsilon R\alpha 2_{197}$, $PcFc_\epsilon R\alpha 3_{199}$ (which are disclosed in the Examples section) and allelic variants thereof. Preferred nucleic acid molecules to encode a $Fc_\epsilon R$ molecules include $ncFc_\epsilon R\alpha 4_{591}$, $ncFc_\epsilon R\alpha 4_{687}$, $ncFc_\epsilon R\alpha 4_{991}$, $ncFc_\epsilon R\alpha 4_{759}$ and allelic variants thereof, as well as $ncFc_\epsilon R\alpha 1_{609}$, $ncFc_\epsilon R\alpha 1_{591}$, $ncFc_\epsilon R\alpha 2_{609}$, $ncFc_\epsilon R\alpha 2_{591}$, $ncFc_\epsilon R\alpha 3_{617}$, $ncFc_\epsilon R\alpha 3_{597}$ (which are disclosed in the Examples section) and allelic variants thereof. A preferred nucleic acid sequence encoding a canine $Fc_\epsilon R$ molecule includes SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, and/or a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences.

An isolated canine $Fc_\epsilon R$ molecule protein of the present invention can be produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell that is capable of expressing the protein, the recombinant cell being produced by transforming a host cell with one or more nucleic acid molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their

ability to be expressed is retained. Suitable and preferred nucleic acid molecules with which to transform a cell are as disclosed herein for suitable and preferred $Fc_\epsilon R$ nucleic acid molecules per se. Particularly preferred nucleic acid molecules to include in recombinant cells of the present invention include $ncFc_\epsilon R\alpha 1_{609}$, $ncFc_\epsilon R\alpha 1_{591}$, $ncFc_\epsilon R\alpha 2_{609}$, $ncFc_\epsilon R\alpha 2_{591}$, $ncFc_\epsilon R\alpha 3_{617}$, $ncFc_\epsilon R\alpha 3_{597}$, $ncFc_\epsilon R\alpha 4_{591}$, $ncFc_\epsilon R\alpha 4_{687}$, $ncFc_\epsilon R\alpha 4_{991}$ and/or $ncFc_\epsilon R\alpha 4_{759}$.

Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule. Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing a canine $Fc_\epsilon R$ molecule protein of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), parasite (including protozoa and ectoparasite), insect, other animal and plant cells.

Preferably, a recombinant cell is transfected with a recombinant molecule of the present invention. A recombinant molecule of the present invention includes at least one of any nucleic acid molecules heretofore described operatively linked to at least one of any transcription control sequence capable of effectively regulating expression of the nucleic acid molecule(s) in the cell to be transformed, examples of which are disclosed herein. Particularly preferred recombinant molecules include pVL-$ncFc_\epsilon R\alpha 4_{591}$, pVL-$ncFc_\epsilon R\alpha 1_{609}$, pVL-$ncFc_\epsilon R\alpha 2_{609}$, and pVL-$ncFc_\epsilon R\alpha 3_{617}$. Details regarding the production of $Fc_\epsilon R$ molecule nucleic acid molecule-containing recombinant molecules are disclosed herein. Particularly preferred recombinant cells of the present invention include *S. frugiperda*:pVL-$ncFc_\epsilon R\alpha 4_{591}$, *Trichoplusia ni*:BV-$ncFc_\epsilon R\alpha 4_{591}$, *S. frugiperda*:pVL-$ncFc_\epsilon R\alpha 1_{609}$, *S. frugiperda*:pVL-$ncFc_\epsilon R\alpha 2_{609}$, *S. frugiperda*:pVL-$ncFc_\epsilon R\alpha 3_{608}$, *Trichoplusia ni*:BV-$ncFc_\epsilon R\alpha 1_{609}$, *Trichoplusia ni*:BV-$ncFc_\epsilon R\alpha 2_{609}$, and *Trichoplusia ni*:BV-$ncFc_\epsilon R\alpha 3_{617}$.

A $Fc_\epsilon R$ molecule of the present invention can include chimeric molecules comprising a portion of a $Fc_\epsilon R$ molecule that binds to an IgE and a second molecule that

enables the chimeric molecule to be bound to a substrate in such a manner that the $Fc_\epsilon R$ portion binds to IgE in essentially the same manner as a $Fc_\epsilon R$ molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of an immunoglobulin molecule.

A canine $Fc_\epsilon R$ molecule of the present invention can be contained in a formulation, herein referred to as a $Fc_\epsilon R$ formulation. For example, a canine $Fc_\epsilon R$ molecule can be combined with a buffer in which the $Fc_\epsilon R$ is solubilized, and/or with a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a $Fc_\epsilon R$ can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate buffer, bicarbonate buffer, HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffered saline), TES buffer (Tris-EDTA buffered saline), Tris buffer and TAE buffer (Tris-acetate-EDTA). Examples of carriers include, but are not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be combined with $Fc_\epsilon R$ or conjugated (i.e., attached) to $Fc_\epsilon R$ in such a manner as to not substantially interfere with the ability of the $Fc_\epsilon R$ to selectively bind to IgE.

A canine $Fc_\epsilon R$ molecule of the present invention can be bound to the surface of a cell expressing the $Fc_\epsilon R$. A preferred $Fc_\epsilon R$-bearing cell includes a recombinant cell expressing a nucleic acid molecule encoding a canine $Fc_\epsilon R$ alpha chain of the present invention. A more preferred recombinant cell of the present invention expresses a nucleic acid molecule that encodes at least one of the following proteins: $PcFc_\epsilon R\alpha 1_{197}$, $PcFc_\epsilon R\alpha 2_{197}$, $PcFc_\epsilon R\alpha 3_{199}$, $PcFc_\epsilon R\alpha 4_{253}$, $PcFc_\epsilon R\alpha 4_{229}$, $PcFc_\epsilon R\alpha 4_{197}$ and $PcFc_\epsilon R\alpha 4_{173}$. An even more preferred recombinant cell expresses a nucleic acid molecule including $ncFc_\epsilon R\alpha 1_{609}$, $ncFc_\epsilon R\alpha 1_{591}$, $ncFc_\epsilon R\alpha 2_{609}$, $ncFc_\epsilon R\alpha 2_{591}$,$ncFc_\epsilon R\alpha 3_{617}$, $ncFc_\epsilon R\alpha 3_{597}$, $ncFc_\epsilon R\alpha 4_{591}$, $ncFc_\epsilon R\alpha 4_{687}$, $ncFc_\epsilon R\alpha 4_{991}$ and $ncFc_\epsilon R\alpha 4_{759}$, or allelic variants thereof, with a recombinant cell expressing a nucleic acid molecule comprising a nucleic acid sequence SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:13 or SEQ ID NO:27, or a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:13 or SEQ ID NO:27, being even more preferred.

In addition, a $Fc_\epsilon R$ formulation of the present invention can include not only a $Fc_\epsilon R$ but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers to any molecule capable of being selectively bound by an antibody. As used herein, specific binding of a first molecule to a second molecule refers to the ability of the first molecule to preferentially bind to (e.g., have higher affinity higher avidity for) the second molecule when compared to the ability of a first molecule to bind to a third molecule. The first molecule need not necessarily be the natural ligand of the second molecule. Examples of antibodies used in the present invention include, but are not limited to, antibodies that bind selectively to the constant region of an IgE heavy chain (i.e., anti-IgE isotype antibodies) or antibodies that bind selectively to an IgE having a specific antigen specificity (i.e., anti-IgE idiotypic antibodies). Examples of antigens used in the present invention include any antigen known to induce the production of IgE. Preferred antigens include allergens and parasite antigens. Allergens of the present invention are preferably derived from fungi, trees, weeds, shrubs, grasses, wheat, corn, soybeans, rice, eggs, milk, cheese, bovines (or cattle), poultry, swine, sheep, yeast, fleas, flies, mosquitos, mites, midges, biting gnats, lice, bees, wasps, ants, true bugs or ticks. A suitable flea allergen includes an allergen derived from a flea, and in particular a flea saliva antigen. Preferred flea saliva antigens include antigens such as those disclosed in PCT Patent Publication No. WO 96/11271, published April 18, 1996, by Frank et al. (this publication is incorporated by reference herein in its entirety), U.S. Patent Application Serial Nos. 08/319,590 (filed Oct. 7, 1994), 08/487,001 (filed June 7, 1995), 08/487,608 (filed June 7, 1995) and 08/630,822 (filed April 10, 1996), with flea saliva products and flea saliva proteins being particularly preferred. According to the present invention, a flea saliva protein includes a protein produced by recombinant DNA methods, as well as proteins isolated by other methods disclosed in PCT Patent Publication No. WO 96/11271, U.S. Patent Application Serial Nos. 08/319,590 (filed Oct. 7, 1994), 08/487,001 (filed June 7, 1995), 08/487,608 (filed June 7, 1995) and 08/630,822 (filed April 10, 1996).

Preferred general allergens include those derived from grass, Meadow Fescue, Curly Dock, plantain, Mexican Firebush, Lamb's Quarters, pigweed, ragweed, sage, elm, cocklebur, Box Elder, walnut, cottonwood, ash, birch, cedar, oak, mulberry, cockroach,

Dermataphagoides, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Mucor*, *Penicillium*, *Pullularia*, *Rhizopus* and/or *Tricophyton*. More preferred general allergens include those derived from Johnson Grass, Kentucky Blue Grass, Meadow Fescue, Orchard Grass, Perennial Rye Grass, Redtop Grass, Timothy Grass, Bermuda Grass, Brome Grass, Curly Dock, English Plantain, Mexican Firebush, Lamb's Quarters, Rough Pigweed Short Ragweed, Wormwood Sage, American Elm, Common Cocklebur, Box Elder, Black Walnut, Eastern Cottonwood, Green Ash, River Birch, Red Cedar, Red Oak, Red Mulberry, Cockroach, *Dermataphagoides farinae*, *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Fusarium vasinfectum*, *Helminthosporium sativum*, *Mucor recemosus*, *Penicillium notatum*, *Pullularia pullulans*, *Rhizopus nigricans* and/or *Tricophyton* spp. Preferred parasite antigens include, but are not limited to, helminth antigens, in particular heartworm antigens, such as Di33 (described in U.S. Patent Application Serial No. 08/715,628, filed September 18, 1996, to Grieve et al.; this publication is incorporated by reference herein in its entirety). The term "derived from" refers to a natural allergen of such plants or organisms (i.e., an allergen directly isolated from such plants or organisms), as well as non-natural allergens of such plants or organisms that possess at least one epitope capable of eliciting an immune response against an allergen (e.g., produced using recombinant DNA technology or by chemical synthesis).

The present invention also includes canine $Fc_\epsilon R$ mimetopes and use thereof to detect IgE. In accordance with the present invention, a "mimetope" refers to any compound that is able to mimic the ability of a canine $Fc_\epsilon R$ molecule to bind to canine IgE. A mimetope can be a peptide that has been modified to decrease its susceptibility to degradation but that still retains IgE-binding activity. Other examples of mimetopes include, but are not limited to, carbohydrate-based compounds, lipid-based compounds, nucleic acid-based compounds, natural organic compounds, synthetically derived organic compounds, anti-idiotypic antibodies and/or catalytic antibodies, or fragments thereof. A mimetope can be obtained by, for example, screening libraries of synthetic compounds for compounds capable of binding to IgE. A mimetope can also be obtained by, for example, rational drug design. In a rational drug design procedure, the three-dimensional structure of a compound of the present invention can be analyzed by, for

example, nuclear magnetic resonance (NMR) or x-ray crystallography. The three-dimensional structure can then be used to predict structures of potential mimetopes by, for example, computer modeling. The predicted mimetope structures can then be produced by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of $Fc_\epsilon R$ mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex® technology, peptides identified by random screening of peptide libraries and proteins identified by phage display technology.

One embodiment of the present invention is a method to detect canine IgE which includes the steps of: (a) contacting an isolated canine Fc_ϵ receptor ($Fc_\epsilon R$) molecule with a putative canine IgE-containing composition under conditions suitable for formation of a $Fc_\epsilon R$ molecule:IgE complex; and (b) detecting levels of IgE by detecting said $Fc_\epsilon R$ molecule:IgE complex. Presence of such a $Fc_\epsilon R$ molecule:IgE complex indicates that the canine is producing IgE. The present method can further include the step of determining whether a canine IgE complexed with a canine $Fc_\epsilon R$ molecule is heat labile. Certain classes of IgE are heat labile when incubated at about 56°C for about 4 hours. Without being bound by theory, Applicants believe that heat labile forms of IgE bind to certain allergens and non-heat labile forms of IgE bind to other types of allergens. As such, detection of heat labile IgE compared with non-heat labile IgE can be used to discriminate between allergen sensitivities. For example, Applicants believe that canine IgE antibodies that bind to certain flea allergens and heartworm allergens are heat labile while canine IgE antibodies that bind to certain plant allergens are not heat labile. Thus, the presence of non-heat labile IgE can indicate that an animal is sensitive to certain plant allergens but not to certain flea or heartworm allergens. Moreover, Applicants believe that identification of heat labile IgE and non-heat labile IgE using a canine $Fc_\epsilon R$ suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE. As such, a $Fc_\epsilon R$ molecule of the present invention may be useful for detecting molecules bound by the $Fc_\epsilon R$ molecule that are not identical to a known IgE.

As used herein, canine refers to any member of the dog family, including domestic dogs, wild dogs and zoo dogs. Examples of dogs include, but are not limited to, domestic dogs, wild dogs, foxes, wolves, jackals and coyotes.

As used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with a canine $Fc_\epsilon R$ molecule. Formation of a complex between a canine $Fc_\epsilon R$ and a canine IgE refers to the ability of the $Fc_\epsilon R$ to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a $Fc_\epsilon R$ of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between a $Fc_\epsilon R$ and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures, reaction times) as well as methods to optimize such conditions are known to those skilled in the art, and examples are disclosed herein. Examples of complex formation conditions are also disclosed in, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989; the reference Sambrook et al., *ibid.*, is incorporated by reference herein in its entirety.

As used herein, the term "detecting complex formation" refers to determining if any complex is formed, i.e., assaying for the presence (i.e., existence) of a complex. If complexes are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between canine $Fc_\epsilon R$ and canine IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in the art (see, for example, Sambrook et al. *ibid.*), examples of which are disclosed herein.

In one embodiment, a putative canine IgE-containing composition of the present method includes a biological sample from a canine. A suitable biological sample includes, but is not limited to, a bodily fluid composition or a cellular composition. A bodily fluid refers to any fluid that can be collected (i.e., obtained) from an animal, examples of which include, but are not limited to, blood, serum, plasma, urine, tears, aqueous humor, central nervous system fluid (CNF), saliva, lymph, nasal secretions, milk and feces. Such a composition of the present method can, but need not be,

pretreated to remove at least some of the non-IgE isotypes of immunoglobulin and/or other proteins, such as albumin, present in the fluid. Such removal can include, but is not limited to, contacting the bodily fluid with a material, such as Protein G, to remove IgG antibodies and/or affinity purifying IgE antibodies from other components of the body fluid by exposing the fluid to, for example, Concanavalin A. In another embodiment, a composition includes collected bodily fluid that is pretreated to concentrate immunoglobulin contained in the fluid. For example, immunoglobulin contained in a bodily fluid can be precipitated from other proteins using ammonium sulfate. A preferred composition of the present method is serum.

In another embodiment, a composition of the present method includes an IgE-producing cell. Such a cell can have IgE bound to the surface of the cell and/or can secrete IgE. Examples of such cells include basophil cells and myeloma cells. IgE can be bound to the surface of a cell, for example by being either bound directly to the membrane of a cells or bound to a molecule (e.g., an antigen) bound to the surface of the cell.

A complex can be detected in a variety of ways including, but not limited to, use of one or more of the following assays: an enzyme-linked immunoassay, a radioimmunoassay, a fluorescence immunoassay, a chemiluminescent assay, a lateral flow assay, an agglutination assay, a particulate-based assay (e.g., using particulates such as, but not limited to, magnetic particles or plastic polymers, such as latex or polystyrene beads), an immunoprecipitation assay, a BioCore™ assay (e.g., using colloidal gold) and an immunoblotting assay (e.g., a western blot). Such assays are well known to those skilled in the art. Assays can be used to give qualitative or quantitative results depending on how they are used. Some assays, such as agglutination, particulate separation, and immunoprecipitation, can be observed visually (e.g., either by eye or by a machine, such as a densitometer or spectrophotometer) without the need for a detectable marker. In other assays, conjugation (i.e., attachment) of a detectable marker to the $Fc_\epsilon R$ or to a reagent that selectively binds to the $Fc_\epsilon R$ or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable markers include, but are not limited to, a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label or a ligand. A ligand refers to a molecule

that binds selectively to another molecule. Preferred detectable markers include, but are not limited to, fluorescein, a radioisotope, a phosphatase (e.g., alkaline phosphatase), biotin, avidin, a peroxidase (e.g., horseradish peroxidase) and biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure® NeutrAvidin®). Preferably, biotin is conjugated to an alpha chain of a $Fc_\epsilon R$. Preferably a carbohydrate group of the $Fc_\epsilon R$ alpha chain is conjugated to biotin.

In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a canine $Fc_\epsilon R$ molecule that is conjugated to a detectable marker. A suitable detectable marker to conjugate to a $Fc_\epsilon R$ molecule includes, but is not limited to, a radioactive label, a fluorescent label, a chemiluminescent label or a chromophoric label. A detectable marker is conjugated to a $Fc_\epsilon R$ molecule or a reagent in such a manner as not to block the ability of the $Fc_\epsilon R$ or reagent to bind to the IgE being detected. Preferably, a carbohydrate group of a $Fc_\epsilon R$ is conjugated to biotin.

In another embodiment, a $Fc_\epsilon R$ molecule:IgE complex is detected by contacting a putative IgE-containing composition with a $Fc_\epsilon R$ molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the $Fc_\epsilon R$ molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, a $Fc_\epsilon R$ molecule, an antigen, an antibody and a lectin, depending upon which portion of the $Fc_\epsilon R$ molecule:IgE complex is being detected. Preferred identifying labeled compounds that are antibodies include, for example, anti-IgE antibodies and anti-$Fc_\epsilon R$ antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a $Fc_\epsilon R$ molecule of the present invention produced in insect cells. An indicator molecule itself can be attached to a detectable marker of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

In one preferred embodiment, a $Fc_\epsilon R$ molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to a $Fc_\epsilon R$ molecule of the present invention. Examples of such a reagent include, but are not limited to, an antibody that selectively binds to a $Fc_\epsilon R$ molecule (referred to herein as an anti-$Fc_\epsilon R$ antibody) or a compound that selectively binds to a detectable marker conjugated to a

$Fc_\epsilon R$ molecule. $Fc_\epsilon R$ molecules conjugated to biotin are preferably detected using streptavidin, more preferably using ImmunoPure® NeutrAvidin® (available from Pierce, Rockford, IL).

In another preferred embodiment, a $Fc_\epsilon R$ molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to an IgE antibody (referred to herein as an anti-IgE reagent). Examples of such an anti-IgE reagent include, but are not limited to, a secondary antibody that is an anti-isotype antibody (e.g., an antibody that selectively binds to the constant region of an IgE), an antibody-binding bacterial surface protein (e.g., Protein A or Protein G), an antibody-binding cell (e.g., a B cell, a T cell, a natural killer cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., an Fc receptor), and an antibody-binding complement protein. Preferred anti-IgE reagents include, but are not limited to, D9 (provided by Doug DeBoer, University of Wisconsin), and CMI antibody #9, CMI antibody #19, CMI antibody #59 and CMI antibody #71 (available from Custom Monoclonal International, West Sacramento, CA). In particular, as used herein, an anti-IgE antibody includes not only a complete antibody but also any subunit or portion thereof that is capable of selectively binding to an IgE heavy chain constant region. For example, a portion of an anti-IgE reagent can include an Fab fragment or a $F(ab')_2$ fragment, which are described in detail in Janeway et al., in *Immunobiology, the Immune System in Health and Disease*, Garland Publishing, Inc., NY, 1996 (which is incorporated herein by this reference in its entirety).

In one embodiment a complex can be formed and detected in solution. In another embodiment, a complex can be formed in which one or more members of the complex are immobilized on (e.g., coated onto) a substrate. Immobilization techniques are known to those skilled in the art. Suitable substrate materials include, but are not limited to, plastic, glass, gel, celluloid, paper, PVDF (poly-vinylidene-fluoride), nylon, nitrocellulose, and particulate materials such as latex, polystyrene, nylon, nitrocellulose, agarose and magnetic resin. Suitable shapes for substrate material include, but are not limited to, a well (e.g., microtiter dish well), a plate, a dipstick, a bead, a lateral flow apparatus, a membrane, a filter, a tube, a dish, a celluloid-type matrix, a magnetic particle, and other particulates. A particularly preferred substrate comprises an ELISA

plate, a dipstick, a radioimmunoassay plate, agarose beads, plastic beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a detectable marker.

A preferred method to detect canine IgE is an immunosorbent assay. An immunoabsorbent assay of the present invention comprises a capture molecule and an indicator molecule. A capture molecule of the present invention binds to an IgE in such a manner that the IgE is immobilized to a substrate. As such, a capture molecule is preferably immobilized to a substrate of the present invention prior to exposure of the capture molecule to a putative IgE-containing composition. An indicator molecule of the present invention detects the presence of an IgE bound to a capture molecule. As such, an indicator molecule preferably is not immobilized to the same substrate as a capture molecule prior to exposure of the capture molecule to a putative IgE-containing composition.

A preferred immunoabsorbent assay method includes a step of either: (a) binding a canine $Fc_\epsilon R$ molecule to a substrate prior to contacting a canine $Fc_\epsilon R$ molecule with a putative IgE-containing composition to form a canine $Fc_\epsilon R$ molecule-coated substrate; or (b) binding a putative canine IgE-containing composition to a substrate prior to contacting a canine $Fc_\epsilon R$ molecule with a putative IgE-containing composition to form a putative IgE-containing composition-coated substrate. Preferably, the substrate is a non-coated substrate, an antigen-coated substrate or an anti-IgE antibody-coated substrate.

Both a capture molecule and an indicator molecule of the present invention are capable of binding to an IgE. Preferably, a capture molecule binds to a different region of an IgE than an indicator molecule, thereby allowing a capture molecule to be bound to an IgE at the same time as an indicator molecule. The use of a reagent as a capture molecule or an indicator molecule depends upon whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, a canine $Fc_\epsilon R$ molecule of the present invention is used as a capture molecule when the $Fc_\epsilon R$ molecule is bound to a substrate. Alternatively, a canine $Fc_\epsilon R$ molecule is used as an indicator molecule when the $Fc_\epsilon R$ molecule is not bound to a substrate. Suitable molecules for use as capture molecules or indicator molecules include, but are not limited to, a canine

$Fc_\epsilon R$ molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

An immunoabsorbent assay of the present invention can further comprise one or more layers and/or types of secondary molecules or other binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a detectable marker) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a detectable marker) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules can be selected by those of skill in the art. Preferred secondary molecules of the present invention include an antigen, an anti-IgE idiotypic antibody and an anti-IgE isotypic antibody. Preferred tertiary molecules can be selected by a skilled artisan based upon the characteristics of the secondary molecule. The same strategy is applied for subsequent layers.

In one embodiment, a desired antigen is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. Preferred antigens include those disclosed herein. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable (i.e., sufficient) to allow for antigen:IgE complex formation bound to the substrate (i.e., IgE in a sample binds to an antigen immobilized on a substrate). Excess non-bound material (i.e., material from the biological sample that has not bound to the antigen), if any, is removed from the substrate under conditions that retain antigen:IgE complex binding to the substrate. Preferred conditions are described generally in Sambrook et al., *ibid.* An indicator molecule that can selectively bind to an IgE bound to the antigen is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the antigen:IgE complex. The indicator molecule can be conjugated to a detectable marker (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand such as of the biotin or avidin family). Excess indicator molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this

embodiment is a canine $Fc_\epsilon R$ molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

In one embodiment, a canine $Fc_\epsilon R$ molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for $Fc_\epsilon R$ molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain $Fc_\epsilon R$ molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the $Fc_\epsilon R$ is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the $Fc_\epsilon R$ molecule:IgE complex. Preferably, the indicator molecule is conjugated to a detectable marker, preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand such as of the biotin or avidin family. Excess indicator molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is an antigen that will bind to IgE in the biological sample or an anti-IgE isotype or idiotype antibody, either preferably being conjugated to fluorescein, an enzyme or biotin.

In one embodiment, an anti-IgE antibody (e.g., isotype- or idiotype-specific antibody) is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. A biological sample collected from a canine is applied to the substrate and incubated under conditions suitable to allow for anti-IgE antibody:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. A canine $Fc_\epsilon R$ molecule is added to the substrate and incubated to allow formation of a complex between the canine $Fc_\epsilon R$ molecule and the anti-IgE antibody:IgE complex. Preferably, the canine $Fc_\epsilon R$ molecule is conjugated to a detectable marker (preferably to biotin, an enzyme label or a fluorescent label). Excess $Fc_\epsilon R$ molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis.

In one embodiment, an immunosorbent assay of the present invention does not utilize a capture molecule. In this embodiment, a biological sample collected from a canine is applied to a substrate, such as a microtiter dish well or a dipstick, and incubated under conditions suitable to allow for IgE binding to the substrate. Any IgE present in the bodily fluid is immobilized on the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A canine $Fc_\epsilon R$ molecule is added to the substrate and incubated to allow formation of a complex between the canine $Fc_\epsilon R$ molecule and canine IgE. Preferably, the $Fc_\epsilon R$ molecule is conjugated to a detectable marker (preferably to biotin, an enzyme label or a fluorescent label). Excess $Fc_\epsilon R$ molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis.

Another preferred method to detect canine IgE is a lateral flow assay, examples of which are disclosed in U.S. Patent No. 5,424,193, issued June 13, 1995, by Pronovost et al.; U.S. Patent No. 5,415,994, issued May 16, 1995, by Imrich et al; WO 94/29696, published December 22, 1994, by Miller et al.; and WO 94/01775, published January 20, 1994, by Pawlak et al.; each of these patent publications is incorporated by reference herein in its entirety. In one embodiment, a biological sample is placed in a lateral flow apparatus that includes the following components: (a) a support structure defining a flow path; (b) a labeling reagent comprising a bead conjugated to an antigen, the labeling reagent being impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising a canine IgE-binding composition. Preferred antigens include those disclosed herein. The capture reagent is located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone. The support structure comprises a material that does not impede the flow of the beads from the labeling zone to the capture zone. Suitable materials for use as a support structure include ionic (i.e., anionic or cationic) material. Examples of such a material include, but are not limited to, nitrocellulose (NC), PVDF and carboxymethylcellulose (CM). The support structure defines a flow path that is lateral and is divided into zones, namely a labeling zone and a capture zone. The apparatus can further comprise a sample receiving zone located along the flow path, more preferably upstream of the labeling

reagent. The flow path in the support structure is created by contacting a portion of the support structure downstream of the capture zone, preferably at the end of the flow path, to an absorbent capable of absorbing excess liquid from the labeling and capture zones.

In this embodiment, the biological sample is applied to the sample receiving zone which includes a portion of the support structure. The labeling zone receives the sample from the sample receiving zone which is directed downstream by the flow path. The labeling zone comprises the labeling reagent that binds to IgE. A preferred labeling reagent is an antigen conjugated, either directly or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a detectable marker, preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample structure also comprises a capture zone downstream of the labeling zone. The capture zone receives labeling reagent from the labeling zone which is directed downstream by the flow path. The capture zone contains the capture reagent, preferably a canine $Fc_{\epsilon}R$ molecule of the present invention that immobilizes canine IgE complexed to the antigen in the capture zone. The capture reagent is preferably fixed to the support structure by drying or lyophilization. The labeling reagent accumulates in the capture zone and the accumulation is assessed visually or by an optical detection device.

In another embodiment, a lateral flow apparatus used to detect canine IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a canine $Fc_{\epsilon}R$ molecule of the present invention, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising an antigen, the capture reagent being located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone. The apparatus preferably also includes a sample receiving zone located along the flow path, preferably upstream of the labeling reagent. The apparatus preferably also includes an absorbent located at the end of the flow path.

One embodiment of the present invention is an inhibition assay in which the presence of canine IgE in a putative canine IgE-containing composition is determined by adding such composition to a canine $Fc_{\epsilon}R$ molecule of the present invention and an

isolated canine IgE known to bind to the $Fc_\epsilon R$ molecule. The absence of binding of the $Fc_\epsilon R$ molecule to the known IgE indicating the presence of IgE in the putative IgE-containing composition.

The present invention also includes kits to detect canine IgE based, for example, on the disclosed detection methods. One embodiment is a kit to detect canine IgE comprising a canine Fc_ϵ receptor ($Fc_\epsilon R$) molecule and a means for detecting a canine IgE. Suitable and preferred canine $Fc_\epsilon R$ molecules are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the canine $Fc_\epsilon R$ molecule or to a canine IgE. A preferred kit of the present invention further comprises a detection means including one or more antigens such as those disclosed herein, an antibody capable of selectively binding to canine IgE such as those disclosed herein and/or a compound capable of binding to a detectable marker conjugated to a canine $Fc_\epsilon R$ molecule (e.g., avidin, streptavidin and ImmunoPure® NeutrAvidin when the detectable marker is biotin).

A preferred embodiment of a kit of the present invention is a flea allergen kit comprising a flea allergen such as those disclosed herein. A particularly preferred flea allergen for use with a flea allergen kit includes a flea saliva product or a flea saliva protein.

Another preferred kit of the present invention is a general allergen kit comprising an allergen common to all regions of the United States and a canine $Fc_\epsilon R$ molecule of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that are found substantially throughout the United States (i.e., essentially not limited to certain regions of the United States). A general allergen kit provides an advantage over regional allergen kits because a single kit can be used to test a canid from any geographical location in the United States. Suitable and preferred general allergens for use with a general allergen kit of the present invention include those general allergens disclosed herein.

Another preferred kit of the present invention is a food allergen kit comprising (a) a food allergen such as beef, chicken, pork, a mixture of fish, such as cod, halibut or and tuna, egg, milk, Brewer's yeast, whole wheat, corn, soybean, cheese and/or rice, and

(b) a canine FcεR molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

A preferred kit of the present invention is one in which the allergen is immobilized to a substrate. If a kit comprises two or more antigens, the kit can comprise one or more compositions, each composition comprising one antigen. As such, each antigen can be tested separately. A kit can also contain two or more diagnostic reagents for detecting canine IgE, additional isolated canine IgE antigens and/or antibodies as disclosed herein. Particularly preferred are kits used in a lateral flow assay format. It is within the scope of the present invention that a lateral flow assay kit can include one or more lateral flow assay apparatuses. Multiple lateral flow apparatuses can be attached to each other at one end of each apparatus, thereby creating a fan-like structure.

In particular, a method and kit of the present invention are useful for diagnosing abnormal conditions in animals that are associated with changing levels of canine IgE. Particularly preferred conditions to diagnose include allergies, parasitic infections and neoplasia. For example, a method and kit of the present invention are particularly useful for detecting flea allergy dermatitis (FAD), when such method or kit includes the use of a flea saliva antigen. FAD is defined as a hypersensitive response to fleabites. Preferably, a putative IgE-containing composition is obtained from an animal suspected of having FAD. In addition, methods and kits of the present invention are particularly useful for detecting helminth infection, in particular heartworm infection, when such methods or kits include the use of a helminth antigen, such as Di33. Preferably, a putative canine IgE-containing composition is obtained from a canine suspected of having a helminth infection.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

It is to be noted that the Examples include a number of molecular biology, microbiology, immunology and biochemistry techniques considered to be known to those skilled in the art. Disclosure of such techniques can be found, for example, in Sambrook et al., *ibid.*, and related references.

Example 1

This example describes the construction of recombinant baculoviruses expressing a truncated portion of the α chain of canine Fc_ϵ receptor.

Recombinant molecules pVL-ncFc$_\epsilon$R$\alpha 1_{609}$, pVL-ncFc$_\epsilon$R$\alpha 2_{609}$, and pVL-ncFc$_\epsilon$R$\alpha 3_{617}$, each containing nucleic acid molecules encoding the extracellular domain of the canine Fc$_\epsilon$R α chain, operatively linked to baculovirus polyhedron transcription control sequences were produced in the following manner. Three different canine Fc$_\epsilon$R α chain extracellular domain nucleic acid molecule-containing fragments, each of about 608 to about 609 nucleotides were amplified by polymerase chain reaction (PCR) from either a canine splenic mononuclear cell cDNA library or a canine lymph node mononuclear cell cDNA library, each library produced using standard techniques, using a forward primer CIERMet containing a *Bam*HI site, having the nucleic acid sequence 5'-TGC GGA TCC AAT ATG CCT GCT TCC ATG GGA G-3' (denoted SEQ ID NO:1) and a reverse primer CIERSec containing an *Eco*RI site, having the nucleic acid sequence 5'-TTG GAA TTC TTA CTC TTT TTT CAC AAT AAT GTT G-3' (denoted herein as SEQ ID NO:2). The resulting PCR products were digested with *Bam*HI and *Eco*RI to produce the following nucleic acid molecules: ncFc$_\epsilon$R$\alpha 1_{609}$ (also denoted ncFc$_\epsilon$RαLN4$_{609}$), ncFc$_\epsilon$R$\alpha 2_{609}$ (also denoted ncFc$_\epsilon$RαSPL6$_{609}$) and ncFc$_\epsilon$R$\alpha 3_{617}$ (also denoted ncFc$_\epsilon$RαSPL3R$_{617}$). Nucleic acid molecule ncFc$_\epsilon$R$\alpha 1_{609}$ was obtained from the PCR reaction derived from the canine lymph node mononuclear cell cDNA library. Nucleic acid molecules ncFc$_\epsilon$R$\alpha 2_{609}$ and ncFc$_\epsilon$R$\alpha 3_{617}$ were obtained from the PCR reaction derived from the canine splenic mononuclear cell cDNA library. Nucleic acid molecules ncFc$_\epsilon$R$\alpha 1_{609}$, ncFc$_\epsilon$R$\alpha 2_{609}$, and ncFc$_\epsilon$R$\alpha 3_{617}$ each were sequenced by the Sanger dideoxy chain termination method, using the PRISM™ Ready Dye Terminator Cycle Sequencing Kit with Ampli Taq DNA Polymerase, FS (available from the Perkin-Elmer Corporation, Norwalk, CT). Nucleic acid molecules ncFc$_\epsilon$R$\alpha 1_{609}$, ncFc$_\epsilon$R$\alpha 2_{609}$, and ncFc$_\epsilon$R$\alpha 3_{617}$ each contained an about 608 to an about 609 nucleotide fragment encoding the extracellular domain of the canine Fc$_\epsilon$R α chain, the coding strands of which have nucleic acid sequences denoted SEQ ID NO:3, SEQ ID NO:8, and SEQ ID NO:13, respectively. The complement of SEQ ID NO:3 is represented herein by SEQ

ID NO:5. The complement of SEQ ID NO:8 is represented herein by SEQ ID NO:10. The complement of SEQ ID NO:13 is represented herein by SEQ ID NO:15.

Translation of SEQ ID NO:3 indicates that nucleic acid molecule $ncFc_\epsilon R\alpha 1_{609}$ encodes a $Fc_\epsilon R$ protein of about 197 amino acids, referred to herein as $PcFc_\epsilon R\alpha 1_{197}$, having amino acid sequence SEQ ID NO:4, assuming an open reading frame having a start codon spanning from about nucleotide 10 through about nucleotide 12 of SEQ ID NO:3 and a stop codon spanning from about nucleotide 601 through about nucleotide 603 of SEQ ID NO:3. This open reading frame, excluding the stop codon, comprises nucleic acid molecule $ncFc_\epsilon R\alpha 1_{591}$ of the present invention, the nucleic acid sequence of which is represented herein by SEQ ID NO:6. The complement of SEQ ID NO:6 is represented herein by SEQ ID NO:7.

Translation of SEQ ID NO:8 indicates that nucleic acid molecule $ncFc_\epsilon R\alpha 2_{609}$ encodes a $Fc_\epsilon R$ protein of about 197 amino acids, referred to herein as $PcFc_\epsilon R\alpha 2_{197}$, having amino acid sequence SEQ ID NO:9, assuming an open reading frame having a start codon spanning from about nucleotide 10 through about nucleotide 12 of SEQ ID NO:8 and a stop codon spanning from about nucleotide 601 through about nucleotide 603 of SEQ ID NO:8. This open reading frame, excluding the stop codon, comprises nucleic acid molecule $ncFc_\epsilon R\alpha 2_{591}$ of the present invention, the nucleic acid sequence of which is represented herein by SEQ ID NO:11. The complement of SEQ ID NO:11 is represented herein by SEQ ID NO:12.

Translation of SEQ ID NO:13 indicates that nucleic acid molecule $ncFc_\epsilon R\alpha 3_{617}$ encodes a $Fc_\epsilon R$ protein of about 199 amino acids, referred to herein as $PcFc_\epsilon R\alpha 3_{199}$, having amino acid sequence SEQ ID NO:14, assuming that the initiation codon spans from about nucleotide 10 through about nucleotide 12 of SEQ ID NO:13 and the last codon spans from about nucleotide 595 through about nucleotide 597 of SEQ ID NO:13. This open reading frame comprises nucleic acid molecule $ncFc_\epsilon R\alpha 3_{597}$ of the present invention, the nucleic acid sequence of which is represented herein by SEQ ID NO:16. The complement of SEQ ID NO:16 is represented herein by SEQ ID NO:17.

In order to produce baculovirus recombinant molecules capable of directing the production of $PcFc_\epsilon R\alpha 1_{197}$, $PcFc_\epsilon R\alpha 2_{197}$, and $PcFc_\epsilon R\alpha 3_{199}$, nucleic acid molecules $ncFc_\epsilon R\alpha 1_{609}$, $ncFc_\epsilon R\alpha 2_{609}$, and $ncFc_\epsilon R\alpha 3_{617}$ were subcloned into unique *Bam*HI and

*Eco*RI sites of pVL1393 baculovirus shuttle plasmid (available from Pharmingen, San Diego, CA) to produce recombinant molecules referred to herein as pVL-ncFc$_\epsilon$Rα1$_{609}$, pVL-ncFc$_\epsilon$Rα2$_{609}$, and pVL-ncFc$_\epsilon$Rα3$_{617}$, respectively. The resultant recombinant molecules pVL-ncFc$_\epsilon$Rα1$_{609}$, pVL-ncFc$_\epsilon$Rα2$_{609}$, and pVL-ncFc$_\epsilon$Rα3$_{617}$ were verified for proper insert orientation by restriction mapping.

Recombinant molecules pVL-ncFc$_\epsilon$Rα1$_{609}$, pVL-ncFc$_\epsilon$Rα2$_{609}$, and pVL-ncFc$_\epsilon$Rα3$_{617}$ were co-transfected with a linear Baculogold™ baculovirus DNA (available from Pharmingen) into *S. frugiperda* Sf9 cells (available from Invitrogen Corp., San Diego, CA) using methods prescribed by the manufacturer to form recombinant cells *S. frugiperda*:pVL-ncFc$_\epsilon$Rα1$_{609}$, *S. frugiperda*:pVL-ncFc$_\epsilon$Rα2$_{609}$, and *S. frugiperda*:pVL-ncFc$_\epsilon$Rα3$_{617}$. Recombinant baculoviruses were plaque purified and amplified from each transfection by methods well known to those skilled in the art, to produce recombinant baculoviruses BV-ncFc$_\epsilon$Rα1$_{609}$, BV-ncFc$_\epsilon$Rα2$_{609}$, and BV-ncFc$_\epsilon$Rα3$_{617}$, respectively.

Example 2

This example describes the production of PcFc$_\epsilon$Rα1$_{197}$, PcFc$_\epsilon$Rα2$_{197}$, and PcFc$_\epsilon$Rα3$_{199}$ canine Fc$_\epsilon$R α chain proteins.

About 1.5 liter cultures of serum-free ex-Cell Medium (available from Invitrogen) were seeded with about 1 x 10^6 *Trichoplusia ni* cells (High Five™ cells; available from Invitrogen) per milliliters (ml) of medium. The cell cultures were inoculated with recombinant baculoviruses BV-ncFc$_\epsilon$Rα1$_{609}$, BV-ncFc$_\epsilon$Rα2$_{609}$, and BV-ncFc$_\epsilon$Rα3$_{617}$, respectively, at multiplicities of infection (MOI) of about 2 to about 5 plaque forming units (pfu) per cell to produce recombinant cells *Trichoplusia ni*-BV-ncFc$_\epsilon$Rα1$_{609}$, *Trichoplusia ni*-BV-ncFc$_\epsilon$Rα2$_{609}$, and *Trichoplusia ni*-BV-ncFc$_\epsilon$Rα3$_{617}$. The infections were allowed to proceed at a controlled temperature of 27°C for 48 hours, to produce recombinant proteins of PcFc$_\epsilon$Rα1$_{197}$, PcFc$_\epsilon$Rα2$_{197}$, and PcFc$_\epsilon$Rα3$_{199}$. Following infection, cells were separated from the medium by centrifugation, and the medium was frozen at -70°C.

Example 3

This example describes the binding of PcFc$_\epsilon$Rα1$_{197}$, PcFc$_\epsilon$Rα2$_{197}$, or PcFc$_\epsilon$Rα3$_{199}$ protein to canine IgE.

About 4.5 ml of the culture media described immediately above containing $PcFc_{\epsilon}R\alpha 1_{197}$, $PcFc_{\epsilon}R\alpha 2_{197}$, or $PcFc_{\epsilon}R\alpha 3_{199}$, respectively, were loaded onto columns comprising a canine IgE monoclonal antibody (a gift from Chris Grant, Custom Monoclonals International, West Sacramento, CA) linked to sepharose 4B. Each column was washed with about 4 ml of carbonate buffer (0.1 M $NaHCO_3$, pH 8.3 and 0.5 M NaCl). Protein bound to the IgE on each column was eluted from the column using about 3 ml of 0.1 M glycine-HCl, pH 2.8. Each column was further washed with about 1 ml of carbonate buffer and then with about 4 ml of buffer comprising 0.1 M $NaHCO_3$, pH 8.3. The elution samples and wash samples from a given column were combined and concentrated to a volume of about 0.35 ml. The eluted protein from each column was resolved on separate 14% Tris-glycine polyacrylamide-SDS gels. The gels were then stained with coomassie stain. A diffused band was observed at about 31 kilodaltons (kD).

Amino (N-) terminal amino acid sequencing analysis was performed on protein contained in the diffused band using standard procedures known to those in the art (see, for example, Geisow et al., 1989, in *Protein Sequencing: A Practical Approach*, JBC Findlay and MJ Geisow (eds.), IRL Press, Oxford, England, pp. 85-98; Hewick et al., 1981, *J. Biol. Chem.*, Vol. 256, pp. 7990-7997). The N-terminal partial amino acid sequence of a protein contained in the band was determined to be S D T L K P T V X M N P P X N L I (as represented in standard single letter code, and denoted herein as SEQ ID NO:18; "X" represents any amino acid). Comparison of SEQ ID NO:18 and the amino acid sequence of the canine $Fc_{\epsilon}R$ alpha chain reported in Hayashi et al., *ibid.*, indicated that $PcFc_{\epsilon}R\alpha 1_{197}$, $PcFc_{\epsilon}R\alpha 2_{197}$, and $PcFc_{\epsilon}R\alpha 3_{199}$, expressed in baculovirus, each bound to canine IgE antibodies.

Example 4

This example describes the isolation, by DNA hybridization, and sequencing of a nucleic acid molecule encoding the $Fc_{\epsilon}R$ α chain from *Canis canis*.

A. Isolation of nucleic acid molecule $ncFc_{\epsilon}R\alpha 4_{991}$

A nucleic acid molecule was isolated from a canine mast cell cDNA library by the molecule's ability to hybridize with a ^{32}P-labeled probe derived from a PCR clone encoding the canine $Fc_{\epsilon}R$ α chain. The canine mast cell cDNA library was prepared

using standard techniques. Using a modification of the protocol described in the cDNA Synthesis Kit, the mast cell cDNA library was screened, using duplicate plaque lifts, with a ^{32}P-labeled probe comprising $ncFc_{\epsilon}R\alpha 1_{609}$ (SEQ ID NO:3). A plaque purified clone containing a canine nucleic acid molecule encoding the $Fc_{\epsilon}R$ α chain was converted into a double stranded recombinant molecule, using the ExAssist™ helper phage and SOLR™ *E. coli* according to the *in vivo* excision protocol described in the ZAP-cDNA Synthesis Kit (available from Stratagene). Double-stranded plasmid DNA was prepared using an alkaline lysis protocol, such as that described in Sambrook et al., *ibid.* The plasmid comprised a canine $Fc_{\epsilon}R$ α chain nucleic acid molecule of about 991 nucleotides denoted herein as $ncFc_{\epsilon}R\alpha 4_{991}$.

B. Sequence analysis of nucleic acid molecule $ncFc_{\epsilon}R\alpha 4_{991}$

The nucleic acid molecule $ncFc_{\epsilon}R\alpha 4_{991}$ was sequenced by standard Sanger dideoxy chain termination sequencing techniques (see, for example, Sambrook et al, *ibid.).* DNA sequence analysis, including the compilation of sequences and the determination of open reading frames, were performed using the MacVector™ program (available from the Eastman Kodak Company, New Haven, CT), or the DNAsis™ program (available from Hitachi Software, San Bruno, CA). Protein sequence analysis, including the determination of molecular weight and isoelectric point (pI) was performed using the MacVector™ program.

The nucleic acid sequence of the coding strand of $ncFc_{\epsilon}R\alpha 4_{991}$ is denoted herein as SEQ ID NO:19. Translation of SEQ ID NO:19 suggests that nucleic acid molecule $ncFc_{\epsilon}R\alpha 4_{991}$ encodes a full-length canine $Fc_{\epsilon}R$ α chain protein of about 253 amino acids, referred to herein as $PcFc_{\epsilon}R\alpha 4_{253}$, having amino acid sequence SEQ ID NO:20, assuming an open reading frame in which the initiation codon spans from about nucleotide 35 through about nucleotide 37 of SEQ ID NO:19 and the termination codon spans from about nucleotide 794 through about nucleotide 796 of SEQ ID NO:19. The complement of SEQ ID NO:20 is represented herein by SEQ ID NO:21. The coding region encoding $PcFc_{\epsilon}R\alpha 4_{253}$, is represented by nucleic acid molecule $ncFc_{\epsilon}R\alpha 4_{759}$, having a coding strand with the nucleic acid sequence represented by SEQ ID NO:22 and a complementary strand with nucleic acid sequence SEQ ID NO:23. The amino acid

sequence of $PcFc_{\epsilon}R\alpha_{253}$ (i.e., SEQ ID NO:21) predicts that $PcFc_{\epsilon}R\alpha_{253}$ has an estimated molecular weight of about 28.5 kD and an estimated pI of about 9.62.

Analysis of SEQ ID NO: 20 suggests the presence of a signal peptide encoded by a stretch of amino acids spanning from about amino acid 1 through about amino acid 24. The proposed mature protein, denoted herein as $PcFc_{\epsilon}R\alpha4_{229}$, contains about 229 amino acids, the sequence of which is shown as SEQ ID NO:24. The coding strand encoding $PcFc_{\epsilon}R\alpha4_{229}$ is represented herein as SEQ ID NO:30. The amino acid sequence of $PcFc_{\epsilon}R\alpha4_{229}$ (i.e., SEQ ID NO:24) predicts that $PcFc_{\epsilon}R\alpha4_{229}$ has an estimated molecular weight of about 26 kD, an estimated pI of about 9.65 and five predicted asparagine-linked glycosylation sites extending from about amino acids 29-31, 42-44, 71-73, 135-137 and 148-150, respectively.

Comparison of amino acid sequence SEQ ID NO:20 with amino acid sequences reported in GenBank indicates that SEQ ID NO:20 showed the most homology, i.e., about 100% identity between SEQ ID NO:20 and a *Canis canis* $Fc_{\epsilon}R$ α chain protein (GenBank accession number D16413). Comparison of amino acid sequence SEQ ID NO:22 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:22 showed the most homology, i.e., about 100% identity between SEQ ID NO:22 and a canine mRNA for $Fc_{\epsilon}R$ α chain (GenBank accession D16413).

Example 5

This Example demonstrates the production of secreted canine FcεR α chain protein in eukaryotic cells.

To produce a secreted form of a canine $Fc_{\epsilon}R$ α chain, recombinant molecule pVL-$ncFc_{\epsilon}R\alpha4_{591}$, containing a canine $Fc_{\epsilon}R$ α chain nucleic acid molecule encoding a secreted form of canine $Fc_{\epsilon}R$ α chain spanning nucleotides from about 35 through about 625 of SEQ ID NO:19 operatively linked to baculovirus polyhedron transcription control sequences, was produced in the following manner. A canine $Fc_{\epsilon}R$ α chain nucleic acid molecule of about 591 nucleotides was PCR amplified from $ncFc_{\epsilon}R\alpha4_{991}$ DNA using a sense primer canIgEr FWD having the nucleic acid sequence 5' GCG **AAG ATC** TAT AAA TAT GCC TGC TTC CAT GGG- 3' (SEQ ID NO:25; *BglII* site shown in bold) and an antisense primer canIgEr REV having the nucleic acid sequence 5' GCA **GGA ATT** CTT ACT CTT TTT TCA CAA TAA TGT -3' (SEQ ID NO:26; *EcoRI* site shown

in bold). The N-terminal primer was designed from the pol h sequence of baculovirus with modifications to enhance expression in the baculovirus system.

The about 591 base pair PCR product (referred to as $ncFc_{\epsilon}R\alpha4_{591}$) has a coding strand nucleic acid sequence denoted herein as SEQ ID NO:27. The complement of SEQ ID NO:27 is represented herein by SEQ ID NO:29. Translation of SEQ ID NO:27 indicates that nucleic acid molecule $ncFc_{\epsilon}R\alpha4_{591}$ encodes a $Fc_{\epsilon}R$ α chain protein of about 197 amino acids, referred to herein as $PcFc_{\epsilon}R\alpha4_{197}$, having amino acid sequence SEQ ID NO:28. Nucleic acid molecule $ncFc_{\epsilon}R\alpha_{591}$ encodes a secretable form of the canine $Fc_{\epsilon}R$ α chain. The processed protein product encoded by $ncFc_{\epsilon}R\alpha4_{591}$ does not possess a leader sequence or transmembrane domain, and is referred to herein as $PcFc_{\epsilon}R\alpha4_{173}$, represented herein by SEQ ID NO:31.

Nucleic acid molecule $Bv\text{-}ncFc_{\epsilon}R\alpha_{591}$ was digested with *BglII* and *EcoRI* and subcloned into the unique *BglII* and *EcoRI* sites of baculovirus shuttle plasmid pVL1392 (available from Pharmingen, San Diego, CA) to produce the recombinant molecule referred to herein as $pVL\text{-}ncFc_{\epsilon}R\alpha_{591}$. The resultant recombinant molecule, $pVL\text{-}ncFc_{\epsilon}R\alpha_{591}$, was verified for proper insert orientation by restriction mapping. The recombinant molecule $pVL\text{-}ncFc_{\epsilon}R\alpha_{591}$ was co-transfected with a Baculogold™ baculovirus DNA into *S. frugiperda* Sf9 cells (available from Invitrogen) to form recombinant cells denoted *S. frugiperda*:$pVL\text{-}ncFc_{\epsilon}R\alpha_{591}$. Recombinant baculovirus was plaque purified and amplified from each transfection by methods well known to those skilled in the art, to produce recombinant baculovirus $BV\text{-}ncFc_{\epsilon}R\alpha_{591}$.

S. frugiperda:$pVL\text{-}ncFc_{\epsilon}R\alpha_{591}$ cells were cultured in order to produce a secreted canine $Fc_{\epsilon}R$ α chain protein, $PcFc_{\epsilon}R\alpha4_{197}$ in the following manner. An about 1.5 liter cultures of serum-free ex-Cell Medium was seeded with about 1 x 10^6 *Trichoplusia ni* cells (High Five™ cells) per ml of medium. The cell culture was inoculated with recombinant baculovirus $BV\text{-}ncFc_{\epsilon}R\alpha_{591}$ at a multiplicity of infection (MOI) of about 2 to about 5 plaque forming units (pfu) per cell to produce recombinant cell *Trichoplusia ni*:$BV\text{-}ncFc_{\epsilon}R\alpha_{591}$. The infection was allowed to proceed at a controlled temperature of 27°C for 48 hours, to produce recombinant protein of $PcFc_{\epsilon}R\alpha_{197}$. Following infection, cells were separated from the medium by centrifugation, and the medium was frozen at -70°C.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Heska Corporation
(B) STREET: 1825 Sharp Point Drive
(C) CITY: Fort Collins
(D) STATE: CO
(E) COUNTRY: US
(F) POSTAL CODE (ZIP): 80525
(G) TELEPHONE: (970) 493-7272
(H) TELEFAX: (970) 484-9505

(ii) TITLE OF INVENTION: METHOD TO DETECT CANINE IgE

(iii) NUMBER OF SEQUENCES: 31

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: LAHIVE & COCKFIELD, LLP
(B) STREET: 28 STATE STREET
(C) CITY: BOSTON
(D) STATE: MA
(E) COUNTRY: US
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: Windows 95
(D) SOFTWARE: ASCII DOS TEXT

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/833,488
(B) FILING DATE: 07-APR-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Rothenberger, Scott D.
(B) REGISTRATION NUMBER: 41,277
(C) REFERENCE/DOCKET NUMBER:

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 227-7400
(B) TELEFAX: (617) 742-4214

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGCGGATCCA ATATGCCTGC TTCCATGGGA 30

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTGGAATTCT TACTCTTTTT TCACAATAAT GTTG 34

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 609 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 10..603

(ix) FEATURES:
(A) NAME/KEY: R = A or G
(B) LOCATION: 187

(ix) FEATURES:
(A) NAME/KEY: Xaa = unknown amino acid
(B) LOCATION: 60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GGATCCAAT ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG          42
          Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu
            1               5                  10

TGG CTA GCG CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA        84
Trp Leu Ala Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser
              15                  20                  25

GAT ACC TTG AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT       126
Asp Thr Leu Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn
                    30                  35

ACA ATA TTG AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG       168
Thr Ile Leu Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly
 40                  45                  50

AAC AAC TCC CTT GAA GTC GRC TCT GCT GTG TGG CTC CAC AAC       210
Asn Asn Ser Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn
      55                  60                  65

AAC ACT ACT TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC AAT       252
Asn Thr Thr Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn
          70                  75                  80
```

```
AAA GCC CAA ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA     294
Lys Ala Gln Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu
                 85                  90                  95

AAT AGA TCC ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC     336
Asn Arg Ser Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe
                100                 105

ACA GAG TGG CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG     378
Thr Glu Trp Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met
110                 115                 120

GAG GGT GAG AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT     420
Glu Gly Glu Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn
    125                 130                 135

TTG AGG CTC ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC     462
Leu Arg Leu Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro
        140                 145                 150

ATC AGG TAC TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC     504
Ile Arg Tyr Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn
            155                 160                 165

GTC ACA ACC AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG     546
Val Thr Thr Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln
                170                 175

ATC CAG CAG AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT     588
Ile Gln Gln Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile
180                 185                 190

GTG AAA AAA GAG TAA GAATTC                                 609
Val Lys Lys Glu
    195
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 197 amino acids
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURES:
(A) NAME/KEY: Xaa = unknown amino acid
(B) LOCATION: 60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
  1               5                  10

Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
 15                  20                  25

Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
     30                  35                  40

Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
         45                  50                  55
```

Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn Asn Thr Thr
60 65 70

Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
75 80

Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
85 90 95

Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
100 105 110

Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
115 120 125

Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
130 135 140

Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
145 150

Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
155 160 165

Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
170 175 180

Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
185 190 195
Glu

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 609 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: Y = G or T
(B) LOCATION: 422

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATTCTTAC TCTTTTTTCA CAATAATGTT GAGGACTTTA GAGGTGTAGC 50
CTTTCTGCTG GATCTGGCCT GAGCAGGAAT AGTTGCCGCT GTTTTTGGTT 100
GTGACGTTGC TAATGGAGAT GTTGAAGTTC TCGTACCAGT ACCTGATGGG 150
GATGCCATCC TTGTAGTAGG TCACCTTTGT GAGCCTCAAA TTCTTCCAAC 200
TATGGCACCT GATGAGGAAG CTCTCACCCT CCATCACCAC GTTGGCAGAG 250
GCTTGAAGGA TCAGCCACTC TGTGAAGACT GTTAGGTACA CAGGATCACT 300
CAGGATGGAT CTATTTTCCC GACACCTGTA CTCCCCACTG TCCTGGATTT 350
GGGCTTTATT GATGTCCAAA CGTGAAGTCG TCTCTTGCAA AGTAGTGTTG 400
TTGTGGAGCC ACACAGCAGA GYCGACTTCA AGGGAGTTGT TCCCAGTACA 450
TGTAAGAGTC ACACTGTCAT CCTTCAATAT TGTATTCCAT GGCGGGTTCA 500
TGGACACTGT AGGTTTCAAG GTATCTGATG ACATGACACC TGGAGAGGAG 550
AGCAGCAGCG CTAGCCACAG CAGGGCAGGG CCTCCCATGG AAGCAGGCAT 600
ATTGGATCC 609

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 591 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 1..591

(ix) FEATURES:
(A) NAME/KEY: R = A or G
(B) LOCATION: 179

(ix) FEATURES:
(A) NAME/KEY: Xaa = unknown amino acid
(B) LOCATION: 60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG TGG CTA GCG      42
Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
 1               5                  10

CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA GAT ACC TTG      84
Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
 15                  20                  25

AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG     126
Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
     30                  35                  40

AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC     168
Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
         45                  50                  55

CTT GAA GTC GRC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT     210
Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn Asn Thr Thr
             60                  65                  70

TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC AAT AAA GCC CAA     252
Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
         75                  80

ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC     294
Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
 85                  90                  95

ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC ACA GAG TGG     336
Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
     100                 105                 110

CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG GAG GGT GAG     378
Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
         115                 120                 125

AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT TTG AGG CTC     420
Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
             130                 135                 140
```

```
ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC ATC AGG TAC     462
Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
                145                 150

TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC GTC ACA ACC     504
Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
155                 160                 165

AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG ATC CAG CAG     546
Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
    170                 175                 180

AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT GTG AAA AAA     588
Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
        185                 190                 195

GAG                                                        591
Glu
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 591 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: Y = C or T
(B) LOCATION: 413

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CTCTTTTTTC ACAATAATGT TGAGGACTTT AGAGGTGTAG CCTTTCTGCT      50
GGATCTGGCC TGAGCAGGAA TAGTTGCCGC TGTTTTTGGT TGTGACGTTG     100
CTAATGGAGA TGTTGAAGTT CTCGTACCAG TACCTGATGG GGATGCCATC     150
CTTGTAGTAG GTCACCTTTG TGAGCCTCAA ATTCTTCCAA CTATGGCACC     200
TGATGAGGAA GCTCTCACCC TCCATCACCA CGTTGGCAGA GGCTTGAAGG     250
ATCAGCCACT CTGTGAAGAC TGTTAGGTAC ACAGGATCAC TCAGGATGGA     300
TCTATTTTCC CGACACCTGT ACTCCCCACT GTCCTGGATT TGGGCTTTAT     350
TGATGTCCAA ACGTGAAGTC GTCTCTTGCA AAGTAGTGTT GTTGTGGAGC     400
CACACAGCAG AGYCGACTTC AAGGGAGTTG TTCCCAGTAC ATGTAAGAGT     450
CACACTGTCA TCCTTCAATA TTGTATTCCA TGGCGGGTTC ATGGACACTG     500
TAGGTTTCAA GGTATCTGAT GACATGACAC CTGGAGAGGA GAGCAGCAGC     550
GCTAGCCACA GCAGGGCAGG GCCTCCCATG GAAGCAGGCA T              591
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 609 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 10..604

(ix) FEATURES:
(A) NAME/KEY: Xaa = unknown amino acid
(B) LOCATION: 60, 195, 196

(ix) FEATURES:
(A) NAME/KEY: K = G or T
(B) LOCATION: 187

(ix) FEATURES:
(A) NAME/KEY: N = unknown nucleotide
(B) LOCATION: 592, 595, 596

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
GGATCCAAT ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG          42
          Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu
            1               5                  10

TGG CTA GCG CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA        84
Trp Leu Ala Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser
             15                  20                  25

GAT ACC TTG AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT       126
Asp Thr Leu Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn
                    30                  35

ACA ATA TTG AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG       168
Thr Ile Leu Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly
 40                  45                  50

AAC AAC TCC CTT GAA GTC GKC TCT GCT GTG TGG CTC CAC AAC       210
Asn Asn Ser Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn
      55                  60                  65

AAC ACT ACT TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC AAT       252
Asn Thr Thr Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn
          70                  75                  80

AAA GCC CAA ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA       294
Lys Ala Gln Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu
             85                  90                  95

AAT AGA TCC ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC       336
Asn Arg Ser Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe
                100                 105

ACA GAG TGG CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG       378
Thr Glu Trp Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met
110                 115                 120

GAG GGT GAG AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT       420
Glu Gly Glu Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn
     125                 130                 135

TTG AGG CTC ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC       462
Leu Arg Leu Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro
          140                 145                 150

ATC AGG TAC TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC       504
Ile Arg Tyr Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn
             155                 160                 165
```

```
GTC ACA ACC AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG      546
Val Thr Thr Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln
                170                 175

ATC CAG CAG AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT      588
Ile Gln Gln Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile
180                 185                 190

GTG NAA NNA GAG TAA GAATTC                                  609
Val Xaa Xaa Glu
    195
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 197 amino acids
(B) TYPE: amino acids
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURES:
(A) NAME/KEY: Xaa = unknown amino acid
(B) LOCATION: 60, 195, 196

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
 1               5                  10

Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
 15                  20                  25

Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
 30                  35                  40

Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
         45                  50                  55

Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn Asn Thr Thr
             60                  65                  70

Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
                 75                  80

Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
 85                  90                  95

Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
100                 105                 110

Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
        115                 120                 125

Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
            130                 135                 140

Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
                145                 150

Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
155                 160                 165
```

Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
170 175 180

Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Xaa Xaa
185 190 195

Glu

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 609 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: N = any nucleotide
(B) LOCATION: 14, 15, 18

(ix) FEATURES:
(A) NAME/KEY: M = A or C
(B) LOCATION: 422

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GAATTCTTAC TCTNNTTNCA CAATAATGTT GAGGACTTTA GAGGTGTAGC 50
CTTTCTGCTG GATCTGGCCT GAGCAGGAAT AGTTGCCGCT GTTTTTGGTT 100
GTGACGTTGC TAATGGAGAT GTTGAAGTTC TCGTACCAGT ACCTGATGGG 150
GATGCCATCC TTGTAGTAGG TCACCTTTGT GAGCCTCAAA TTCTTCCAAC 200
TATGGCACCT GATGAGGAAG CTCTCACCCT CCATCACCAC GTTGGCAGAG 250
GCTTGAAGGA TCAGCCACTC TGTGAAGACT GTTAGGTACA CAGGATCACT 300
CAGGATGGAT CTATTTTCCC GACACCTGTA CTCCCCACTG TCCTGGATTT 350
GGGCTTTATT GATGTCCAAA CGTGAAGTCG TCTCTTGCAA AGTAGTGTTG 400
TTGTGGAGCC ACACAGCAGA GMCGACTTCA AGGGAGTTGT TCCCAGTACA 450
TGTAAGAGTC ACACTGTCAT CCTTCAATAT TGTATTCCAT GGCGGGTTCA 500
TGGACACTGT AGGTTTCAAG GTATCTGATG ACATGACACC TGGAGAGGAG 550
AGCAGCAGCG CTAGCCACAG CAGGGCAGGG CCTCCCATGG AAGCAGGCAT 600
ATTGGATCC 609

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 591 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: Xaa = unknown amino acid
(B) LOCATION: 60, 195, 196

(ix) FEATURES:
(A) NAME/KEY: K = G or T
(B) LOCATION: 179

(ix) FEATURES:
(A) NAME/KEY: N = unknown nucleotide
(B) LOCATION: 583, 586, 587

(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 1..591

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG TGG CTA GCG      42
Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
 1               5                  10

CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA GAT ACC TTG      84
Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
 15                  20                  25

AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG     126
Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
     30                  35                  40

AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC     168
Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
         45                  50                  55

CTT GAA GTC GKC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT     210
Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn Asn Thr Thr
             60                  65                  70

TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC AAT AAA GCC CAA     252
Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
         75                  80

ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC     294
Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
 85                  90                  95

ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC ACA GAG TGG     336
Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
     100                 105                 110

CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG GAG GGT GAG     378
Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
         115                 120                 125

AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT TTG AGG CTC     420
Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
             130                 135                 140

ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC ATC AGG TAC     462
Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
         145                 150

TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC GTC ACA ACC     504
Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
 155                 160                 165

AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG ATC CAG CAG     546
Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
     170                 175                 180

AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT GTG NAA NNA     588
Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Xaa Xaa
         185                 190                 195

GAG                                                        591
Glu
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 591 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: N = any nucleotide
(B) LOCATION: 5, 6, 9

(ix) FEATURES:
(A) NAME/KEY: M = A or C
(B) LOCATION: 413

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
CTCTNNTTNC ACAATAATGT TGAGGACTTT AGAGGTGTAG CCTTTCTGCT      50
GGATCTGGCC TGAGCAGGAA TAGTTGCCGC TGTTTTTGGT TGTGACGTTG     100
CTAATGGAGA TGTTGAAGTT CTCGTACCAG TACCTGATGG GGATGCCATC     150
CTTGTAGTAG GTCACCTTTG TGAGCCTCAA ATTCTTCCAA CTATGGCACC     200
TGATGAGGAA GCTCTCACCC TCCATCACCA CGTTGGCAGA GGCTTGAAGG     250
ATCAGCCACT CTGTGAAGAC TGTTAGGTAC ACAGGATCAC TCAGGATGGA     300
TCTATTTTCC CGACACCTGT ACTCCCCACT GTCCTGGATT TGGGCTTTAT     350
TGATGTCCAA ACGTGAAGTC GTCTCTTGCA AAGTAGTGTT GTTGTGGAGC     400
CACACAGCAG AGMCGACTTC AAGGGAGTTG TTCCCAGTAC ATGTAAGAGT     450
CACACTGTCA TCCTTCAATA TTGTATTCCA TGGCGGGTTC ATGGACACTG     500
TAGGTTTCAA GGTATCTGAT GACATGACAC CTGGAGAGGA GAGCAGCAGC     550
GCTAGCCACA GCAGGGCAGG GCCTCCCATG GAAGCAGGCA T              591
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 617 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 10..606

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GGATCCAAT ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG       42
          Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu
           1               5                  10

TGG CTA GCG CTG CTG CTC TCC TCT CCA GGT GTC GTG TCA TCA     84
Trp Leu Ala Leu Leu Leu Ser Ser Pro Gly Val Val Ser Ser
            15                  20                  25

GAT ACC TTG AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT    126
Asp Thr Leu Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn
                30                  35

ACA ATA TTG AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG    168
Thr Ile Leu Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly
 40                  45                  50
```

```
AAC AAC TCC CTT GAA GTC GAC TCT GCT GTG TGG CTC CAC AAC      210
Asn Asn Ser Leu Glu Val Asp Ser Ala Val Trp Leu His Asn
     55                  60                  65

AAC ACT ACT TTG CAA GAG ACG ACT TCA CGT TTG AAC ATC AAT      252
Asn Thr Thr Leu Gln Glu Thr Thr Ser Arg Leu Asn Ile Asn
         70                  75                  80

AAA GCC CAA ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA      294
Lys Ala Gln Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu
             85                  90                  95

AAT AGA TCC ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC      336
Asn Arg Ser Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe
        100                 105

ACA GAG TGG CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG      378
Thr Glu Trp Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met
110                 115                 120

GAG GGT GAG AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT      420
Glu Gly Glu Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn
    125                 130                 135

TTG AGG CTC ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC      462
Leu Arg Leu Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro
        140                 145                 150

ATC AGG TAC TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC      504
Ile Arg Tyr Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn
            155                 160                 165

GTC ACA ACC AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG      546
Val Thr Thr Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln
                170                 175

ATC CAG CAG AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT      588
Ile Gln Gln Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile
180                 185                 190

GTG AAA AAG AGT AAG AAT TCTAAGAATT C                        617
Val Lys Lys Ser Lys Asn
    195
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 199 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
 1               5                  10

Leu Leu Leu Ser Ser Pro Gly Val Val Ser Ser Asp Thr Leu
15                  20                  25

Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
     30                  35                  40
```

Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
45 50 55

Leu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr
60 65 70

Leu Gln Glu Thr Thr Ser Arg Leu Asn Ile Asn Lys Ala Gln
75 80

Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
85 90 95

Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
100 105 110

Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
115 120 125

Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
130 135 140

Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
145 150

Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
155 160 165

Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
170 175 180

Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
185 190 195

Ser Lys Asn

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 617 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAATTCTTAG AATTCTTACT CTTTTTCACA ATAATGTTGA GGACTTTAGA 50
GGTGTAGCCT TTCTGCTGGA TCTGGCCTGA GCAGGAATAG TTGCCGCTGT 100
TTTTGGTTGT GACGTTGCTA ATGGAGATGT TGAAGTTCTC GTACCAGTAC 150
CTGATGGGGA TGCCATCCTT GTAGTAGGTC ACCTTTGTGA GCCTCAAATT 200
CTTCCAACTA TGGCACCTGA TGAGGAAGCT CTCACCCTCC ATCACCACGT 250
TGGCAGAGGC TTGAAGGATC AGCCACTCTG TGAAGACTGT TAGGTACACA 300
GGATCACTCA GGATGGATCT ATTTTCCCGA CACCTGTACT CCCCACTGTC 350
CTGGATTTGG GCTTTATTGA TGTTCAAACG TGAAGTCGTC TCTTGCAAAG 400
TAGTGTTGTT GTGGAGCCAC ACAGCAGAGT CGACTTCAAG GGAGTTGTTC 450
CCAGTACATG TAAGAGTCAC ACTGTCATCC TTCAATATTG TATTCCATGG 500
CGGGTTCATG GACACTGTAG GTTTCAAGGT ATCTGATGAC ACGACACCTG 550
GAGAGGAGAG CAGCAGCGCT AGCCACAGCA GGGCAGGGCC TCCCATGGAA 600
GCAGGCATAT TGGATCC 617

(2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 597 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 1..597

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG TGG CTA GCG      42
Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
 1               5                  10

CTG CTG CTC TCC TCT CCA GGT GTC GTG TCA TCA GAT ACC TTG      84
Leu Leu Leu Ser Ser Pro Gly Val Val Ser Ser Asp Thr Leu
 15                  20                  25

AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG     126
Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
      30                  35                  40

AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC     168
Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
          45                  50                  55

CTT GAA GTC GAC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT     210
Leu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr
              60                  65                  70

TTG CAA GAG ACG ACT TCA CGT TTG AAC ATC AAT AAA GCC CAA     252
Leu Gln Glu Thr Thr Ser Arg Leu Asn Ile Asn Lys Ala Gln
                  75                  80

ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC     294
Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
 85                  90                  95

ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC ACA GAG TGG     336
Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
     100                 105                 110

CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG GAG GGT GAG     378
Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
         115                 120                 125

AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT TTG AGG CTC     420
Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
             130                 135                 140

ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC ATC AGG TAC     462
Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
                 145                 150

TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC GTC ACA ACC     504
Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
155                 160                 165
```

AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG ATC CAG CAG 546
Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
170 175 180

AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT GTG AAA AAG 588
Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
185 190 195

AGT AAG AAT 597
Ser Lys Asn

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 597 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATTCTTACTC TTTTTCACAA TAATGTTGAG GACTTTAGAG GTGTAGCCTT 50
TCTGCTGGAT CTGGCCTGAG CAGGAATAGT TGCCGCTGTT TTTGGTTGTG 100
ACGTTGCTAA TGGAGATGTT GAAGTTCTCG TACCAGTACC TGATGGGGAT 150
GCCATCCTTG TAGTAGGTCA CCTTTGTGAG CCTCAAATTC TTCCAACTAT 200
GGCACCTGAT GAGGAAGCTC TCACCCTCCA TCACCACGTT GGCAGAGGCT 250
TGAAGGATCA GCCACTCTGT GAAGACTGTT AGGTACACAG GATCACTCAG 300
GATGGATCTA TTTTCCCGAC ACCTGTACTC CCCACTGTCC TGGATTTGGG 350
CTTTATTGAT GTTCAAACGT GAAGTCGTCT CTTGCAAAGT AGTGTTGTTG 400
TGGAGCCACA CAGCAGAGTC GACTTCAAGG GAGTTGTTCC CAGTACATGT 450
AAGAGTCACA CTGTCATCCT TCAATATTGT ATTCCATGGC GGGTTCATGG 500
ACACTGTAGG TTTCAAGGTA TCTGATGACA CGACACCTGG AGAGGAGAGC 550
AGCAGCGCTA GCCACAGCAG GGCAGGGCCT CCCATGGAAG CAGGCAT 597

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURES:
(A) NAME/KEY: Xaa = any amino acid
(B) LOCATION: 9, 14

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ser Asp Thr Leu Lys Pro Thr Val Xaa Met Asn Pro Pro Xaa
1 5 10

Asn Leu Ile
15

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 991 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 35..796

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
CTCCAGTCCA GTCGTACGTG GGGGCCACGA GGAG ATG CCT GCT                    43
                                        Met Pro Ala
                                          1

TCC ATG GGA GGC CCT GCC CTG CTG TGG CTA GCG CTG CTG CTC              85
Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala Leu Leu Leu
  5                  10                  15

TCC TCT CCA GGT GTC ATG TCA TCA GAT ACC TTG AAA CCT ACA             127
Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu Lys Pro Thr
        20                  25                  30

GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG AAG GAT GAC             169
Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu Lys Asp Asp
            35                  40                  45

AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC CTT GAA GTC             211
Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser Leu Glu Val
                50                  55

GAC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT TTG CAA GAG             253
Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr Leu Gln Glu
 60                  65                  70

ACG ACT TCA CGT TTG GAC ATC AAT AAA GCC CAA ATC CAG GAC             295
Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln Ile Gln Asp
  75                  80                  85

AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC ATC CTG AGT             337
Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser Ile Leu Ser
        90                  95                  100

GAT CCT GTG TAC CTA ACA GTC TTC ACA GAG TGG CTG ATC CTT             379
Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp Leu Ile Leu
            105                 110                 115

CAA GCC TCT GCC AAC GTG GTG ATG GAG GGT GAG AGC TTC CTC             421
Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu Ser Phe Leu
                120                 125

ATC AGG TGC CAT AGT TGG AAG AAT TTG AGG CTC ACA AAG GTG             463
Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu Thr Lys Val
130                 135                 140

ACC TAC TAC AAG GAT GGC ATC CCC ATC AGG TAC TGG TAC GAG             505
Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr Trp Tyr Glu
  145                 150                 155

AAC TTC AAC ATC TCC ATT AGC AAC GTC ACA ACC AAA AAC AGC             437
Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr Lys Asn Ser
        160                 165                 170

GGC AAC TAT TCC TGC TCA GGC CAG ATC CAG CAG AAA GGC TAC             589
Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln Lys Gly Tyr
            175                 180                 185
```

```
ACC TCT AAA GTC CTC AAC ATT ATT GTG AAA AAA GAG CCC ACC     631
Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys Glu Pro Thr
                190                 195

AAG CAA AAC AAG TAC TCC GGG CTA CAA TTC CTG ATC CCG TTG     673
Lys Gln Asn Lys Tyr Ser Gly Leu Gln Phe Leu Ile Pro Leu
200                 205                 210

GTG GTG GTG ATT CTG TTT GCT GTG GAC ACA GGA CTG TTT ATC     715
Val Val Val Ile Leu Phe Ala Val Asp Thr Gly Leu Phe Ile
    215                 220                 225

TCG ACC AAG CAG CAG TTG ACA GTG CTC TTG CAG ATT AAG AGG     757
Ser Thr Lys Gln Gln Leu Thr Val Leu Leu Gln Ile Lys Arg
        230                 235                 240

ACC AGG AAG AAC AAA AAG CCA GAA CCC GGA AAG AAC TGA         796
Thr Arg Lys Asn Lys Lys Pro Glu Pro Gly Lys Asn
            245                 250

TGCCGCTGCT TAAGAAACAT CAGCATCAGC AATCGCTTCT CCATCGTCAG      846
ACGCAGCTCA CGATGCACAC GGGAAGGTCT GCAGTCATGG CTTTGCAGAA      896
CTGCTTCATT CAACCAACTC AAACTGATTA AGTGGCATGT GATAGTAGGT      946
GCTCAATAAA CGGCAGTTAG ATAAATAAAA AAAAAAAAAA AAAAA           991
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 253 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
 1               5                  10

Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
15                  20                  25

Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
     30                  35                  40

Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
         45                  50                  55

Leu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr
             60                  65                  70

Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
                 75                  80

Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
 85                  90                  95

Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
    100                 105                 110

Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
        115                 120                 125
```

Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
130 135 140

Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
145 150

Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
155 160 165

Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
170 175 180

Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
185 190 195

Glu Pro Thr Lys Gln Asn Lys Tyr Ser Gly Leu Gln Phe Leu
200 205 210

Ile Pro Leu Val Val Val Ile Leu Phe Ala Val Asp Thr Gly
215 220

Leu Phe Ile Ser Thr Lys Gln Gln Leu Thr Val Leu Leu Gln
225 230 235

Ile Lys Arg Thr Arg Lys Asn Lys Lys Pro Glu Pro Gly Lys
240 245 250

Asn

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 991 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TTTTTTTTTT TTTTTTTTTA TTTATCTAAC TGCCGTTTAT TGAGCACCTA 50
CTATCACATG CCACTTAATC AGTTTGAGTT GGTTGAATGA AGCAGTTCTG 100
CAAAGCCATG ACTGCAGACC TTCCCGTGTG CATCGTGAGC TGCGTCTGAC 150
GATGGAGAAG CGATTGCTGA TGCTGATGTT TCTTAAGCAG CGGCATCAGT 200
TCTTTCCGGG TTCTGGCTTT TTGTTCTTCC TGGTCCTCTT AATCTGCAAG 250
AGCACTGTCA ACTGCTGCTT GGTCGAGATA AACAGTCCTG TGTCCACAGC 300
AAACAGAATC ACCACCACCA ACGGGATCAG GAATTGTAGC CCGGAGTACT 350
TGTTTTGCTT GGTGGGCTCT TTTTTCACAA TAATGTTGAG GACTTTAGAG 400
GTGTAGCCTT TCTGCTGGAT CTGGCCTGAG CAGGAATAGT TGCCGCTGTT 450
TTTGGTTGTG ACGTTGCTAA TGGAGATGTT GAAGTTCTCG TACCAGTACC 500
TGATGGGGAT GCCATCCTTG TAGTAGGTCA CCTTTGTGAG CCTCAAATTC 550
TTCCAACTAT GGCACCTGAT GAGGAAGCTC TCACCCTCCA TCACCACGTT 600
GGCAGAGGCT TGAAGGATCA GCCACTCTGT GAAGACTGTT AGGTACACAG 650
GATCACTCAG GATGGATCTA TTTTCCCGAC CCTGTACTC CCCACTGTCC 700
TGGATTTGGG CTTTATTGAT GTCCAAACGT GAAGTCGTCT CTTGCAAAGT 750
AGTGTTGTTG TGGAGCCACA CAGCAGAGTC GACTTCAAGG GAGTTGTTCC 800
CAGTACATGT AAGAGTCACA CTGTCATCCT TCAATATTGT ATTCCATGGC 850
GGGTTCATGG ACACTGTAGG TTTCAAGGTA TCTGATGACA TGACACCTGG 900
AGAGGAGAGC AGCAGCGCTA GCCACAGCAG GGCAGGGCCT CCCATGGAAG 950
CAGGCATCTC CTCGTGGCCC CCACGTACGA CTGGACTGGA G 991

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 759 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 1..759

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG TGG CTA GCG      42
Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
  1               5                  10

CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA GAT ACC TTG      84
Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
 15                  20                  25

AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG     126
Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
      30                  35                  40

AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC     168
Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
          45                  50                  55

CTT GAA GTC GAC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT     210
Leu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr
              60                  65                  70

TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC AAT AAA GCC CAA     252
Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
                  75                  80

ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC     294
Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
 85                  90                  95

ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC ACA GAG TGG     336
Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
     100                 105                 110

CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG GAG GGT GAG     378
Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
         115                 120                 125

AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT TTG AGG CTC     420
Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
             130                 135                 140

ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC ATC AGG TAC     462
Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
                 145                 150

TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC GTC ACA ACC     504
Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
155                 160                 165
```

```
AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG ATC CAG CAG     546
Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
    170                 175                 180

AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT GTG AAA AAA     588
Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
        185                 190                 195

GAG CCC ACC AAG CAA AAC AAG TAC TCC GGG CTA CAA TTC CTG     630
Glu Pro Thr Lys Gln Asn Lys Tyr Ser Gly Leu Gln Phe Leu
            200                 205                 210

ATC CCG TTG GTG GTG GTG ATT CTG TTT GCT GTG GAC ACA GGA     672
Ile Pro Leu Val Val Val Ile Leu Phe Ala Val Asp Thr Gly
                215                 220

CTG TTT ATC TCG ACC AAG CAG CAG TTG ACA GTG CTC TTG CAG     714
Leu Phe Ile Ser Thr Lys Gln Gln Leu Thr Val Leu Leu Gln
225                 230                 235

ATT AAG AGG ACC AGG AAG AAC AAA AAG CCA GAA CCC GGA AAG     756
Ile Lys Arg Thr Arg Lys Asn Lys Lys Pro Glu Pro Gly Lys
    240                 245                 250

AAC                                                        759
Asn
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 759 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
GTTCTTTCCG GGTTCTGGCT TTTTGTTCTT CCTGGTCCTC TTAATCTGCA      50
AGAGCACTGT CAACTGCTGC TTGGTCGAGA TAAACAGTCC TGTGTCCACA     100
GCAAACAGAA TCACCACCAC CAACGGGATC AGGAATTGTA GCCCGGAGTA     150
CTTGTTTTGC TTGGTGGGCT CTTTTTTCAC AATAATGTTG AGGACTTTAG     200
AGGTGTAGCC TTTCTGCTGG ATCTGGCCTG AGCAGGAATA GTTGCCGCTG     250
TTTTTGGTTG TGACGTTGCT AATGGAGATG TTGAAGTTCT CGTACCAGTA     300
CCTGATGGGG ATGCCATCCT TGTAGTAGGT CACCTTTGTG AGCCTCAAAT     350
TCTTCCAACT ATGGCACCTG ATGAGGAAGC TCTCACCCTC CATCACCACG     400
TTGGCAGAGG CTTGAAGGAT CAGCCACTCT GTGAAGACTG TTAGGTACAC     450
AGGATCACTC AGGATGGATC TATTTTCCCG ACACCTGTAC TCCCCACTGT     500
CCTGGATTTG GGCTTTATTG ATGTCCAAAC GTGAAGTCGT CTCTTGCAAA     550
GTAGTGTTGT TGTGGAGCCA CACAGCAGAG TCGACTTCAA GGGAGTTGTT     600
CCCAGTACAT GTAAGAGTCA CACTGTCATC CTTCAATATT GTATTCCATG     650
GCGGGTTCAT GGACACTGTA GGTTTCAAGG TATCTGATGA CATGACACCT     700
GGAGAGGAGA GCAGCAGCGC TAGCCACAGC AGGGCAGGGC CTCCCATGGA     750
AGCAGGCAT                                                 759
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 229 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Ser Asp Thr Leu Lys Pro Thr Val Ser Met Asn Pro Pro Trp
1 5 10

Asn Thr Ile Leu Lys Asp Asp Ser Val Thr Leu Thr Cys Thr
15 20 25

Gly Asn Asn Ser Leu Glu Val Asp Ser Ala Val Trp Leu His
30 35 40

Asn Asn Thr Thr Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile
45 50 55

Asn Lys Ala Gln Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg
60 65 70

Glu Asn Arg Ser Ile Leu Ser Asp Pro Val Tyr Leu Thr Val
75 80

Phe Thr Glu Trp Leu Ile Leu Gln Ala Ser Ala Asn Val Val
85 90 95

Met Glu Gly Glu Ser Phe Leu Ile Arg Cys His Ser Trp Lys
100 105 110

Asn Leu Arg Leu Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile
115 120 125

Pro Ile Arg Tyr Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser
130 135 140

Asn Val Thr Thr Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly
145 150

Gln Ile Gln Gln Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile
155 160 165

Ile Val Lys Lys Glu Pro Thr Lys Gln Asn Lys Tyr Ser Gly
170 175 180

Leu Gln Phe Leu Ile Pro Leu Val Val Val Ile Leu Phe Ala
185 190 195

Val Asp Thr Gly Leu Phe Ile Ser Thr Lys Gln Gln Leu Thr
200 205 210

Val Leu Leu Gln Ile Lys Arg Thr Arg Lys Asn Lys Lys Pro
215 220

Glu Pro Gly Lys Asn
225

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GCGAAGATCT ATAAATATGC CTGCTTCCAT GGG 33

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GCAGGAATTC TTACTCTTTT TTCACAATAA TGT 33

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 591 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 1..591

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG TGG CTA GCG      42
Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
 1               5                  10

CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA GAT ACC TTG      84
Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
 15                  20                  25

AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG     126
Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
      30                  35                  40

AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC     168
Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
          45                  50                  55

CTT GAA GTC GAC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT     210
Leu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr
              60                  65                  70

TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC AAT AAA GCC CAA     252
Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
          75                  80

ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC     294
Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
 85                  90                  95
```

```
ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC ACA GAG TGG     336
Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
    100                 105                 110

CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG GAG GGT GAG     378
Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
    115                 120                 125

AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT TTG AGG CTC     420
Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
            130                 135                 140

ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC ATC AGG TAC     462
Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
        145                 150

TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC GTC ACA ACC     504
Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
155                 160                 165

AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG ATC CAG CAG     546
Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
    170                 175                 180

AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT GTG AAA AAA     588
Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
        185                 190                 195

GAG                                                        591
Glu
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 196 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
 1               5                  10

Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
     15                  20                  25

Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
         30                  35                  40

Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
             45                  50                  55

eu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr
                 60                  65

Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
 70                  75                  80

Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
     85                  90                  95
```

Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
100 105 110

Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
115 120 125

Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
130 135

Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
140 145 150

Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
155 160 165

Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
170 175 180

Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
185 190 195

Glu

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 591 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CTCTTTTTTC ACAATAATGT TGAGGACTTT AGAGGTGTAG CCTTTCTGCT 50
GGATCTGGCC TGAGCAGGAA TAGTTGCCGC TGTTTTTGGT TGTGACGTTG 100
CTAATGGAGA TGTTGAAGTT CTCGTACCAG TACCTGATGG GGATGCCATC 150
CTTGTAGTAG GTCACCTTTG TGAGCCTCAA ATTCTTCCAA CTATGGCACC 200
TGATGAGGAA GCTCTCACCC TCCATCACCA CGTTGGCAGA GGCTTGAAGG 250
ATCAGCCACT CTGTGAAGAC TGTTAGGTAC ACAGGATCAC TCAGGATGGA 300
TCTATTTTCC CGACACCTGT ACTCCCCACT GTCCTGGATT TGGGCTTTAT 350
TGATGTCCAA ACGTGAAGTC GTCTCTTGCA AAGTAGTGTT GTTGTGGAGC 400
CACACAGCAG AGTCGACTTC AAGGGAGTTG TTCCCAGTAC ATGTAAGAGT 450
CACACTGTCA TCCTTCAATA TTGTATTCCA TGGCGGGTTC ATGGACACTG 500
TAGGTTTCAA GGTATCTGAT GACATGACAC CTGGAGAGGA GAGCAGCAGC 550
GCTAGCCACA GCAGGGCAGG GCCTCCCATG GAAGCAGGCA T 591

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 687 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
(A) NAME/KEY: CDS
(B) LOCATION: 1..687

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
TCA GAT ACC TTG AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG      42
Ser Asp Thr Leu Lys Pro Thr Val Ser Met Asn Pro Pro Trp
  1               5                  10

AAT ACA ATA TTG AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT      84
Asn Thr Ile Leu Lys Asp Asp Ser Val Thr Leu Thr Cys Thr
 15                  20                  25

GGG AAC AAC TCC CTT GAA GTC GAC TCT GCT GTG TGG CTC CAC     126
Gly Asn Asn Ser Leu Glu Val Asp Ser Ala Val Trp Leu His
      30                  35                  40

AAC AAC ACT ACT TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC     168
Asn Asn Thr Thr Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile
          45                  50                  55

AAT AAA GCC CAA ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG     210
Asn Lys Ala Gln Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg
              60                  65                  70

GAA AAT AGA TCC ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC     252
Glu Asn Arg Ser Ile Leu Ser Asp Pro Val Tyr Leu Thr Val
                  75                  80

TTC ACA GAG TGG CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG     294
Phe Thr Glu Trp Leu Ile Leu Gln Ala Ser Ala Asn Val Val
 85                  90                  95

ATG GAG GGT GAG AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG     336
Met Glu Gly Glu Ser Phe Leu Ile Arg Cys His Ser Trp Lys
      100                 105                 110

AAT TTG AGG CTC ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC     378
Asn Leu Arg Leu Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile
          115                 120                 125

CCC ATC AGG TAC TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC     420
Pro Ile Arg Tyr Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser
              130                 135                 140

AAC GTC ACA ACC AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC     462
Asn Val Thr Thr Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly
                  145                 150

CAG ATC CAG CAG AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT     504
Gln Ile Gln Gln Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile
155                 160                 165

ATT GTG AAA AAA GAG CCC ACC AAG CAA AAC AAG TAC TCC GGG     546
Ile Val Lys Lys Glu Pro Thr Lys Gln Asn Lys Tyr Ser Gly
      170                 175                 180

CTA CAA TTC CTG ATC CCG TTG GTG GTG GTG ATT CTG TTT GCT     588
Leu Gln Phe Leu Ile Pro Leu Val Val Val Ile Leu Phe Ala
          185                 190                 195

GTG GAC ACA GGA CTG TTT ATC TCG ACC AAG CAG CAG TTG ACA     630
Val Asp Thr Gly Leu Phe Ile Ser Thr Lys Gln Gln Leu Thr
              200                 205                 210
```

GTG CTC TTG CAG ATT AAG AGG ACC AGG AAG AAC AAA AAG CCA 672
Val Leu Leu Gln Ile Lys Arg Thr Arg Lys Asn Lys Lys Pro
215 220

GAA CCC GGA AAG AAC 687
Glu Pro Gly Lys Asn
225

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 173 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ser Asp Thr Leu Lys Pro Thr Val Ser Met Asn Pro Pro Trp
1 5 10

Asn Thr Ile Leu Lys Asp Asp Ser Val Thr Leu Thr Cys Thr
15 20 25

Gly Asn Asn Ser Leu Glu Val Asp Ser Ala Val Trp Leu His
30 35 40

Asn Asn Thr Thr Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile
45 50 55

Asn Lys Ala Gln Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg
60 65 70

Glu Asn Arg Ser Ile Leu Ser Asp Pro Val Tyr Leu Thr Val
75 80

Phe Thr Glu Trp Leu Ile Leu Gln Ala Ser Ala Asn Val Val
85 90 95

Met Glu Gly Glu Ser Phe Leu Ile Arg Cys His Ser Trp Lys
100 105 110

Asn Leu Arg Leu Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile
115 120 125

Pro Ile Arg Tyr Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser
130 135 140

Asn Val Thr Thr Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly
145 150

Gln Ile Gln Gln Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile
155 160 165

Ile Val Lys Lys Glu
170

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.

What is claimed is:

1. A method to detect canine IgE comprising:

(a) contacting an isolated canine Fc_ϵ receptor ($Fc_\epsilon R$) molecule with a putative canine IgE-containing composition under conditions suitable for formation of a $Fc_\epsilon R$ molecule:IgE complex; and

(b) determining the presence of IgE by detecting said $Fc_\epsilon R$ molecule:IgE complex, the presence of said $Fc_\epsilon R$ molecule:IgE complex indicating the presence of IgE.

2. A kit for detecting IgE comprising a canine $Fc_\epsilon R$ molecule and a means for detecting canine IgE.

3. A method to detect canine flea allergy dermatitis comprising:

(a) immobilizing a flea allergen on a substrate;

(b) contacting said flea allergen with a putative canine IgE-containing composition under conditions suitable for formation of an antigen:IgE complex bound to said substrate;

(c) removing non-bound material from said substrate under conditions that retain antigen:IgE complex binding to said substrate; and

(d) detecting the presence of said antigen:IgE complex by contacting said antigen:IgE complex with a canine $Fc_\epsilon R$ molecule.

4. A kit for detecting flea allergy dermatitis comprising a canine Fc_ϵ receptor molecule and a flea allergen.

5. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule comprises at least a portion of a $Fc_\epsilon R$ alpha chain that binds to canine IgE.

6. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule comprises a protein selected from the group consisting of $PcFc_\epsilon R\alpha 1_{197}$, $PcFc_\epsilon R\alpha 2_{197}$, $PcFc_\epsilon R\alpha 3_{199}$, $PcFc_\epsilon R\alpha 4_{253}$, $PcFc_\epsilon R\alpha 4_{229}$, $PcFc_\epsilon R\alpha 4_{173}$ and $PcFc_\epsilon R\alpha 4_{197}$.

7. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule is encoded by a nucleic acid molecule selected from the group consisting of $ncFc_\epsilon R\alpha 1_{609}$, $ncFc_\epsilon R\alpha 1_{591}$, $ncFc_\epsilon R\alpha 2_{609}$, $ncFc_\epsilon R\alpha 2_{591}$, $ncFc_\epsilon R\alpha 3_{617}$, $ncFc_\epsilon R\alpha 3_{597}$, $ncFc_\epsilon R\alpha 4_{591}$, $ncFc_\epsilon R\alpha 4_{687}$, $ncFc_\epsilon R\alpha 4_{991}$ and $ncFc_\epsilon R\alpha 4_{759}$.

8. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule is encoded by a nucleic acid molecule selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27 and SEQ ID NO:30, and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences.

9. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule is conjugated to a detectable marker.

10. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule is conjugated to a detectable marker selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a ligand.

11. The invention of Claims 1 or 3, wherein said putative canine IgE-containing composition comprises a bodily fluid selected from the group consisting of serum, blood and plasma.

12. The method of Claim 1 further comprising the step selected from the group consisting of: binding said canine $Fc_\epsilon R$ molecule to a substrate prior to performing step (a) to form a $Fc_\epsilon R$ molecule-coated substrate; and binding said putative IgE-containing composition to a substrate prior to performing step (a) to form a putative IgE-containing composition-coated substrate, wherein said substrate to be bound to said composition is selected from the group consisting of a non-coated substrate, an antigen-coated substrate and an anti-IgE antibody-coated substrate.

13. The method of Claim 12, wherein said antigen is selected from the group consisting of an allergen and a parasitic antigen.

14. The invention of Claims 13 or 30, wherein said allergen is derived from material selected from the group consisting of fungi, trees, weeds, shrubs, grasses, wheat, corn, soybean, rice, eggs, milk, cheese, bovine, poultry, swine, sheep, yeast, fleas, flies, mosquitos, mites, midges, biting gnats, lice, bees, wasps, ants, true bugs and ticks.

15. The invention of Claims 3 or 4, wherein said flea allergen is a flea saliva antigen.

16. The invention of Claims 3 or 4, wherein said flea allergen is selected from the group consisting of flea saliva products and flea saliva proteins.

17. The method of Claim 12, further comprising removing non-bound material from said antigen-coated substrate or said antibody-coated substrate under conditions that retain antigen or antibody binding to said substrate.

18. The method of Claim 12 or 33, wherein said substrate comprises an ELISA plate, a dipstick, a radioimmunoassay plate, agarose beads, plastic beads, latex beads, immunoblot membranes and immunoblot papers.

19. The invention of Claims 12 or 32, wherein said substrate is latex beads.

20. The method of Claim 1, wherein said step of detecting comprises performing assays selected from the group consisting of enzyme-linked immunoassays, radioimmunoassays, immunoprecipitations, fluorescence immunoassays, chemiluminescent assay, immunoblot assays, lateral flow assays, agglutination assays and particulate-based assays.

21. The method of Claim 1, wherein said step of detecting comprises:

(a) contacting said canine $Fc_\epsilon R$ molecule:IgE complex with an indicator molecule that binds selectively to said $Fc_\epsilon R$ molecule:IgE complex;

(b) removing substantially all of said indicator molecule that does not selectively bind to $Fc_\epsilon R$ molecule:IgE complex; and

(c) detecting said indicator molecule, wherein presence of said indicator molecule is indicative of the presence of IgE.

22. The method of Claim 21, wherein said indicator molecule comprises a compound selected from the group consisting of a $Fc_\epsilon R$ molecule, an antigen, an antibody and a lectin.

23. The method of Claim 1, said method comprising the steps of:

(a) immobilizing said canine $Fc_\epsilon R$ molecule on a substrate;

(b) contacting said canine $Fc_\epsilon R$ molecule with said putative IgE-containing composition under conditions suitable for formation of a $Fc_\epsilon R$ molecule:IgE complex bound to said substrate;

(c) removing non-bound material from said substrate under conditions that retain $Fc_\epsilon R$ molecule:IgE complex binding to said substrate; and

(d) detecting the presence of said $Fc_\epsilon R$ molecule:IgE complex.

24. The method of Claim 23, wherein the presence of said $Fc_\epsilon R$ molecule:IgE complex is detected by contacting said $Fc_\epsilon R$ molecule:IgE complex with a compound selected from the group consisting of an antigen and an antibody that binds selectively to IgE.

25. The method of Claim 24, wherein said compound comprises a detectable marker.

26. The method of Claim 1, said method comprising the steps of:

(a) immobilizing a desired antigen on a substrate;

(b) contacting said antigen with said putative IgE-containing composition under conditions suitable for formation of an antigen:IgE complex bound to said substrate;

(c) removing non-bound material from said substrate under conditions that retain antigen:IgE complex binding to said substrate; and

(d) detecting the presence of said antigen:IgE complex by contacting said antigen:IgE complex with said canine $Fc_\epsilon R$ molecule.

27. The method of Claim 1, said method comprising the steps of:

(a) immobilizing an antibody that binds selectively to IgE on a substrate;

(b) contacting said antibody with said putative IgE-containing composition under conditions suitable for formation of an antibody:IgE complex bound to said substrate;

(c) removing non-bound material from said substrate under conditions that retain antibody:IgE complex binding to said substrate; and

(d) detecting the presence of said antibody:IgE complex by contacting said antibody:IgE complex with said canine $Fc_\epsilon R$ molecule.

28. The method of Claim 1, said method comprising the steps of:

(a) immobilizing said putative IgE-containing composition on a substrate;

(b) contacting said composition with said canine $Fc_\epsilon R$ molecule under conditions suitable for formation of a $Fc_\epsilon R$ molecule:IgE complex bound to said substrate;

(c) removing non-bound material from said substrate under conditions that retain $Fc_\epsilon R$ molecule:IgE complex binding to said substrate; and

(d) detecting the presence of said $Fc_\epsilon R$ molecule:IgE complex.

29. The method of Claim 28, wherein said canine $Fc_\epsilon R$ molecule comprises a detectable marker.

30. The kit of Claim 2, wherein said detection means further comprises an antigen selected from the group consisting of an allergen and a parasite antigen, wherein said antigen induces IgE antibody production in canids.

31. The kit of Claim 2, wherein said detection means comprises an antibody that selectively binds to canine IgE.

32. The kit of Claim 2, wherein said detection means detects said canine $Fc_\epsilon R$ molecule.

33. The kit of Claim 30, wherein said antigen is immobilized on a substrate.

34. The kit of Claim 30, wherein said parasite antigen is a heartworm antigen.

35. The kit of Claim 2 further comprising an apparatus comprising:

(a) a support structure defining a flow path;

(b) a labeling reagent comprising a bead conjugated to said antigen, wherein said labeling reagent is impregnated within the support structure in a labeling zone; and

(c) a capture reagent comprising said $Fc_\epsilon R$ molecule, wherein said capture reagent is located downstream of said labeling reagent within a capture zone fluidly connected to said labeling zone in such a manner that said labeling reagent can flow from said labeling zone into said capture zone.

36. The kit of Claim 35, wherein said apparatus further comprises a sample receiving zone located along said flow path.

37. The kit of Claim 35, wherein said apparatus further comprises an absorbent located at the end of said flow path.

38. The kit of Claim 36, wherein said sample receiving zone is located upstream of said labeling reagent.

39. The kit of Claim 35, wherein said bead comprises a latex bead.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 98/06774

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/566 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 97 20859 A (IDEXX LAB INC) 12 June 1997 see claims 6,7,10-14,16-27,29,30 see page 1, line 13 - line 21 see page 6, line 15 - line 16 see page 15, line 3 - line 16 see page 29, line 12 - line 25 ---	1-39
X	WO 95 16203 A (GENENTECH INC ;TAI WAI FEI DAVID (US); LOWE JOHN (US); JARDIEU PAU) 15 June 1995 see claims 1,8,14-16 see page 4, line 18 - page 6, line 9 see page 12, line 25 - page 13, line 5 see page 13, line 14 - line 19 see page 23, line 9 - page 24, line 38 --- -/--	1-39

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 July 1998	28/07/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Routledge, B

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INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 98/06774

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication.where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 29696 A (QUIDEL CORP) 22 December 1994 cited in the application see claims see the whole document	20-39

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INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/US 98/06774

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